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PEANUT-WILT CAUSED BY SCLEROTIUM ROLFSII

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INTRODUCTION

Wolf [1] records a fruitrot of peanuts (*Arachis hypogaea*) caused by *Sclerotium Rolfsii* Sacc., but states (p. 143) that "usually there is no indication of the disease in the appearance of the aboveground parts." During the summer of 1915 the writer observed wilted peanut plants on two plots of peanuts at the Virginia Truck Experiment Station, at Norfolk, Va. The associated organism was found to be *S. Rolfsii*, and its pathogenicity has been established.

Observations are here presented on the life history of the causal organism and experiments on control measures by the use of resistant varieties of peanuts during the season of 1915 and 1916. Data relative to the relation of *S. Rolfsii* to other crops used on the experimental plots are also given.

HISTORY OF THE DISEASE

In 1910 an experiment was started at the Virginia Truck Experiment Station to determine the value of rotation as compared with the continuous planting of peanuts. This experiment, which is still being conducted, occupies four adjoining plots, each about one-third of an acre in size.

Plots 1 and 2 were planted to peanuts in 1910. The next year plots 1 and 3 were planted to peanuts, while plots 2 and 4 were planted to the other crops used in the rotation. In 1912, plots 1 and 4 were in peanuts, the other two being planted to the other rotation crops. In 1913, the 3-year rotation had been completed, so peanuts again occupied plots 1 and 2.

The source of the peanut seed used in beginning this experiment was not known, but during the years 1911 and 1912 peanuts from the previous year's crop were used for seed.

Table I shows the position of the four plots, with their respective crops for the years 1910 to 1916, inclusive.

[1] Wolf, F. A. Leaf spot and some fruit rots of peanut. Ala. Agr. Exp. Sta. Bul. 180, p. 127–150, 5 pl. 1914.

TABLE I.—*Crops grown on plots 1 to 4 from 1910 to 1916, inclusive*

Year.	Plot 1 (continuous planting).	Plot 2 (rotation).	Plot 3 (rotation).	Plot 4 (rotation).
1910	Peanuts....	Peanuts.....	Cowpeas, fall potatoes.	Corn, crimson clover.
1911	.....do.....	Corn, crimson clover.	Peanuts.....	Cowpeas, fall potatoes.
1912	.....do.....	Cowpeas, fall potatoes.	Corn, crimson clover.	Peanuts.
1913	.....do.....	Peanuts.....	Cowpeas, fall potatoes.	Corn, crimson clover.
1914	.....do.....	Corn, crimson clover.	Peanuts.....	Cowpeas, fall potatoes.
1915	.....do.....	Cowpeas, fall potatoes.	Corn, crimson clover.	Peanuts.
1916	.....do.....	Peanuts.....	Cowpeas, fall potatoes.	Corn, crimson clover.

From Mr. W. R. Beattie, formerly of the Office of Horticultural Investigations of the United States Department of Agriculture, it was learned that peanut leafspot, caused by *Cercospora personata*, was the only disease observed during the years from 1910 to 1912.

In 1913, a fresh supply of peanut seed of the Valencia variety was obtained from a concern in Norfolk which buys peanuts in large quantities from various States for making peanut butter and confections. The seed for each succeeding year was saved from peanuts grown on the experimental plots the previous year.

It was learned that a few wilted peanut plants were observed in the 1913 crop and that the number of diseased plants increased each year.

In 1916, the writer planted in experimental plots 1 and 2 Valencia peanuts which were obtained from the Department of Agriculture. Some of these seeds were also planted in a plot near by which had not grown peanuts for at least several years. Over 15 per cent of the plants in the experimental plots wilted, while all of the plants in the plots near by remained healthy. These data, together with the observation of the wilted plants in 1913 and each succeeding year, lead the writer to conclude that the wilt-producing organism was not present in the soil previous to 1913, but had been introduced with the fresh supply of seed used that year.

DESCRIPTION OF THE DISEASE

Under field conditions in Virginia this peanut-wilt begins to show when the plants are between 1 and 2 months old and continues to develop on new plants, here and there, throughout the season. In some cases one or two shoots wilt, while the rest of the shoots appear healthy, but in the majority of cases the whole plant eventually wilts, as shown in Plate 96, *A*. Examination of the wilted shoots generally discloses coarse white mycelium and small brownish sclerotia about the size of

mustard seed near the base of the wilted shoots (Pl. 96, *B*). On sectioning the basal portion of diseased shoots it is found that the tissues are discolored and decaying, indicating that the wilting of these shoots is caused by cutting off the water supply near the base.

Abundant white mycelium and brownish sclerotia also developed on peanut leaves which dropped to the soil about the base of wilted plants. Plate 97, *A*, shows peanut leaflets taken from the surface of the soil under a wilted plant. Thirty-seven sclerotia have been counted on a single leaflet; and in many cases sclerotia developed in greater numbers about the base of wilted shoots, thus providing ample means for reproducing and spreading the organism in subsequent seasons.

Although not as commonly observed, the coarse white mycelium and sclerotia may also be found on both the outside and inside of pods on wilted plants.

During the seasons of 1915 and 1916 wilted plants were observed scattered over the entire area of each plot, indicating that the organism was well distributed throughout the soil. In some cases, however, the plants in adjoining hills wilted in a progressive manner, as though the fungus spread from centers of infection. Plate 97, *B*, shows three such hills, the one in the foreground being infected first. The fungus then apparently spreads to the second hill 12 inches distant, and then to the third hill, where a portion of one plant had been attacked and had wilted at the time the photograph was taken.

SERIOUSNESS OF THE DISEASE

As a result of counts made in 1915 it was found that 15 per cent of the plants had one or more wilted shoots. Similar counts in 1916 showed that the percentage of plants having one or more wilted shoots had increased.

Shoots attacked early in the season produce no peanuts, and those infected after peanuts have formed generally become so badly diseased that the fruits fail to mature. The diseased shoots are so withered and shrunken that they are of little or no value for hay; therefore, wilted plants are practically a total loss.

PATHOGENICITY AND IDENTITY OF THE ASSOCIATED ORGANISM

As coarse white mycelium and small brownish sclerotia were constantly associated with the wilted plants, it seemed advisable to obtain pure cultures of this fungus for identification. Basal sections from numerous diseased shoots were externally disinfected in alcohol and in mercuric chlorid (1:1,000) and plated in nutrient agar. In five days coarse white mycelium had grown out from most of the sections. Transfers of this mycelium to tubes of corn meal and bean pods grew rapidly and eventually produced brownish sclerotia similar to but somewhat larger than those found on the wilted peanut plants. Other fungi grew out from

some plated sections of wilted shoots, but none of these had white mycelium, and all failed to produce the brownish sclerotia.

Healthy peanut plants of various ages were inoculated by placing pure cultures of the various fungi in contact with normal and with wounded stems just below the surface of the soil. Similar plants were labeled to serve as checks. The plants inoculated with the white mycelium and sclerotia began to wilt within a period varying from 15 days to 2 months after inoculation, while the control plants remained healthy. White mycelium and brownish sclerotia developed at the base of artificially infected plants. The causal organism was reisolated from the wilted stems and found to be the same as the original isolations. Plants inoculated with various other fungi isolated from diseased peanut shoots remained healthy.

Isolations of the causal organism were made from time to time during the seasons of 1915 and 1916, and the fungus producing the coarse white mycelium and brownish sclerotia was found to be constantly associated with the wilted peanut shoots.

The appearance of the peanut-wilt fungus corresponded so closely to descriptions of *S. Rolfsii* that the writer tentatively identified the fungus as this organism, although he had not seen any report of this fungus being found in Virginia. The fact that Wolf[1] had, in 1914, reported *S. Rolfsii* as being the cause of a fruitrot of peanuts in Alabama added weight to the writer's suspicion that the peanut-wilt organism found in Virginia is *S. Rolfsii*. During the winter of 1915–16 cultures of the peanut-wilt organism were compared with stock cultures of *S. Rolfsii* from two authentic sources, and the organisms were found to be identical as regards mycelial development, and the size, shape, and color of the sclerotia, thus indicating that this peanut-wilt fungus is *S. Rolfsii*. In January, 1916, the opportunity was afforded the writer to examine specimens of peanut stems infected with *S. Rolfsii* which had been collected at Lykesland, S. C., in 1909 by Dr. W. A. Orton, of the United States Department of Agriculture. They were similar to specimens collected in Virginia by the writer during the season of 1915 and convinced him that the Virginia peanut-wilt is caused by *S. Rolfsii*.

IMPORTANCE OF SEED AS A MEANS OF SPREADING SCLEROTIUM ROLFSII

Having observed that peanut pods from diseased plants bore mycelium and sclerotia of *S. Rolfsii* both externally and internally, the writer deemed it advisable to test the importance of seed and its accompanying trash as a means of transmitting the peanut-wilt fungus.

Seed for the bulk of the 1916 planting was hand-shelled from the 1915 crop by laborers who made no attempt to discriminate between good

[1] Wolf, F. A. Op. cit.

and bad seed. On the same day that the above seed was planted by the laborers the writer planted in each plot one row of seed which he had carefully selected from healthy pods of the 1915 crop. Notes were taken throughout the season, and it was found that the rows planted to carefully selected healthy seed had as many wilted plants as the average of the rows planted with unselected seed. The writer does not think that these data prove that seed is unimportant in spreading *S. Rolfsii*, but it does indicate that the soil of both plots was well infested with this organism. The writer anticipates conducting further experiments along this line during the season of 1917.

From what has been observed of the hand-shelling of peanuts for seed, it seems reasonable to suspect that *S. Rolfsii* may be carried by sclerotia and bits of infected pods which become mixed with the shelled seed. This danger would be greatly lessened by fanning the shelled seed to remove the trash, or by obtaining heavy clean seed from a reliable storage plant which has suitable facilities for thorough cleaning.

EFFECT OF ROTATION ON THE DISEASE

With many fungus diseases a 3-year rotation would be effective in reducing the amount of disease on the susceptible crop, but from the data in Table II it will be seen that this is not true in the case of peanut-wilt.

By reference to Table I, it will be seen that in 1916 peanuts were again planted on plots 1 and 2, which were the plots planted to peanuts in 1913, the year when the peanut-wilt was first observed. Since 1913, plot 1 has been continuously in peanuts, while plot 2 has grown crops of corn and crimson clover in 1914 and a fall crop of Irish potatoes in 1915. When again planted to peanuts in 1916, plot 2 had a somewhat larger percentage of wilted plants than plot 1. There is a possibility that the wilt in plot 2 in 1916 was due to infection introduced with the seed and trash; but the fact that healthy Valencia seed planted by the writer in these two plots also showed a high percentage of disease would indicate that a rotation of more than three years is necessary to free a soil from *S. Rolfsii*.

VARIETAL RESISTANCE TO SCLEROTIUM ROLFSII

Through the courtesy of Mr. F. E. Miller and Mr. H. C. Thompson, of the Office of Horticultural Investigations, United States Department of Agriculture, the following varieties of peanuts were obtained: Tennessee Red, Valencia, Spanish, African, Virginia Bunch, Virginia Runner, and Hog Goober (*Worandzia subterranea*).

To serve as controls, one row of each of these varieties was planted on land which had not previously been planted to peanuts. To test their varietal resistance to *S. Rolfsii*, from a half to a full row of the different varieties was planted in each plot, using every sixth row. These seed

were planted at the same time as the Valencia seed used in the rotation experiment, and the resulting plants were all given the same treatment as regards cultivation. Notes were taken throughout the season and the results are given in Tables II and III.

TABLE II.—*Resistance of various varieties of peanuts to Sclerotium Rolfsii compared with Station Valencia seed on adjoining rows on plot 1*

Variety.	Row.	Number of hills.	Number of wilted hills.	Variety.	Row.	Number of hills.	Number of wilted hills.
Station Valencia	5	66	7	Station Valencia	17	66	11
Government Valencia	6	66	7	Virginia Bunch	18	66	6
Station Valencia	7	66	11	Station Valencia	19	66	8
Do	5	66	7	Do	17	66	10
Tennessee Red	6	66	2	Virginia Runner	18	66	.....
Station Valencia	7	66	11	Station Valencia	19	66	8
Do	11	66	14	Do	23	132	7
Spanish	12	66	7	African	24	130	.....
Station Valencia	13	66	16	Station Valencia	25	132	12
Do	11	66	14				
Hog Goober	12	40	.....				
Station Valencia	13	66	13				

From the data it is seen that Valencia peanuts from Station seed and from Government seed are about equally susceptible to *S. Rolfsii*. Tennessee Red peanuts are considerably more resistant than Valencia. Spanish peanuts are slightly less susceptible than Valencia. Hog Goober is practically immune to *S. Rolfsii*. Virginia Bunch is more resistant than Valencia. Virginia Runner is practically immune. African is practically immune.

TABLE III.—*Resistance of varieties compared with Station Valencia on adjoining rows in plot 2*

Variety.	Row.	Number of hills.	Number of wilted hills.	Variety.	Row.	Number of hills.	Number of wilted hills.
Station Valencia	5	132	29	Station Valencia	17	132	17
Government Valencia	6	132	20	Spanish	18	140	15
Station Valencia	7	132	31	Station Valencia	19	132	29
Do	11	66	14	Do	23	66	13
Tennessee Red	12	66	2	Virginia Bunch	24	66	.....
Station Valencia	13	66	8	Station Valencia	25	66	15
Do	11	66	14	Do	23	66	12
African	12	66	.....	Virginia Runner	24	66	.....
Station Valencia	13	66	8	Station Valencia	25	66	14

The results obtained on plot 2 and presented in Table III substantiate those of plot 1, and indicate that the African, Virginia Runner, Hog Goober, Virginia Bunch, Tennessee Red, and Spanish varieties are resistant to *S. Rolfsii* about in the order named, and all are more resistant than Valencia.

Thompson and Bailey [1] classify Valencia and Tennessee Red peanuts as the same variety, but the writer noted sufficient difference in resistance to *S. Rolfsii* to make it advisable to consider these two as separate varieties from a pathological viewpoint.

As the Virginia Runner, Virginia Bunch, and Spanish varieties are the three varieties grown in Virginia, there is little to be feared from the attacks of *S. Rolfsii* in commercial fields in this State. If, however, this organism should get established in a soil used for peanuts, it would seem advisable to grow resistant varieties in preference to carrying on a rotation of more than three years in order to starve it out.

EFFECT OF SCLEROTIUM ROLFSII ON CRIMSON CLOVER USED AS A COVER CROP

Wolf [2] mentions cowpeas and crimson clover as succumbing to artificial inoculations with *S. Rolfsii*. The crimson clover grown for a winter cover crop on the experimental peanut plots at Norfolk is generally planted in September or October and turned under in May. Under these field conditions in Virginia the writer has observed no infection of crimson clover during 1915 and 1916.

SUMMARY

(1) In 1915 a wilt of peanut plants was found in experimental plots at the Virginia Truck Experiment Station.

(2) The causal organism was probably introduced into the plots in 1913 with a fresh supply of Valencia seed imported that year.

(3) The disease under field conditions appeared when the plants were from 1 to 2 months old and continued to develop throughout the season.

(4) The wilting was due to the fungus attacking the shoots at or near the surface of the soil and killing the invaded tissues. White mycelium and brownish sclerotia about the size of mustard seed were found about the base of wilted peanut stems and also on peanut leaves which had dropped to the soil about the base of wilted plants. Mycelium and sclerotia were also found associated both externally and internally on the pods on diseased plants.

(5) Wilted plants were scattered over the entire area of each plot, thus indicating that the causal organism was well distributed. In some cases several adjoining hills were observed to wilt in a progressive way, which indicated the spreading of the fungus from one hill to the one adjoining in the same row.

(6) Isolations from diseased shoots and inoculations with pure cultures of the associated organisms established the pathogenicity of the fungus producing the coarse white mycelium and brownish sclerotia. Comparisons of pure cultures of this fungus with descriptions and stock

[1] Thompson, H. C., and Bailey, H. S. Peanut oil. U. S. Dept. Agr. Farmers' Bul. 751, 16 p. 1916.
[2] Wolf, F. A. Op. cit., p. 144-145.

cultures of *Sclerotium Rolfsii* established the identity of the peanut-wilt fungus as this organism.

(7) Counts of wilted plants in 1915 showed that 15 per cent had one or more diseased shoots. Similar counts in 1916 showed a larger percentage of wilted plants. No fruits are produced by shoots attacked early in the season, and shoots attacked later in the season generally fail to mature their fruits. Diseased shoots are dry and shrunken and of little or no value for hay.

(8) Preliminary tests indicated that the careful selection of healthy seed did not reduce the amount of wilt; probably due to the fact that the soil was thoroughly infested with *S. Rolfsii*.

(9) Data relative to the value of a 3-year rotation indicate that *S. Rolfsii* lives over in the soil for three years and produces as large a percentage of disease on land where a 3-year rotation was practiced as on land continuously planted to peanuts.

(10) Tests of six varieties of peanuts and one hog goober, *Worandzia subterranea*, planted in soil badly infested with *S. Rolfsii* showed that the Valencia peanut is most susceptible to this organism and that Spanish, Tennessee Red, and Virginia Bunch are respectively resistant in the order named, while Virginia Runner, African, and Hog Goober are practically immune to *S. Rolfsii*.

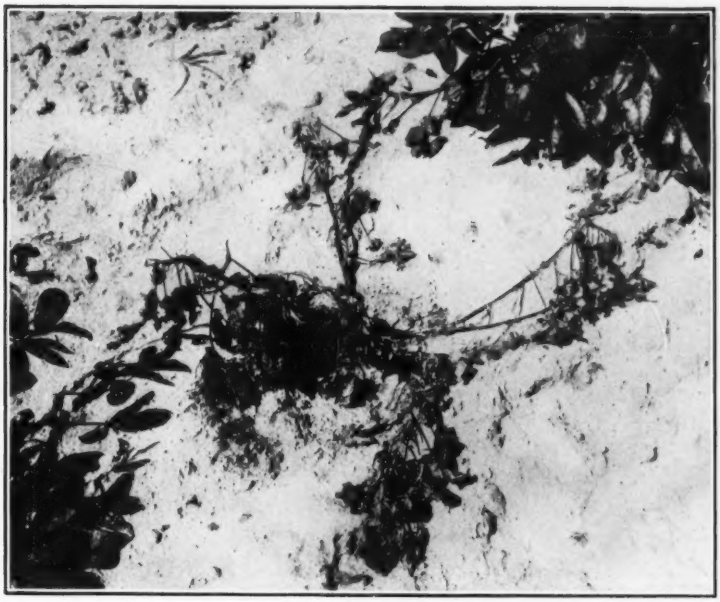
(11) Experiments indicate that soil infested with *Sclerotium Rolfsii* should be planted to resistant varieties of peanuts in preference to attempting to rid the soil of this parasite by rotation.

(12) Observations during the seasons of 1915 and 1916 indicate that *S. Rolfsii* does not attack crimson clover planted in September or October for a cover crop.

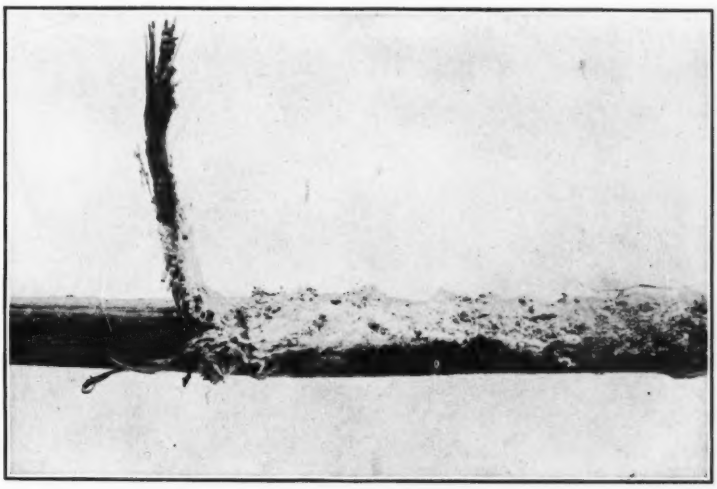
PLATE 96

A.—A hill of peanut plants completely wilted by *Sclerotium Rolfsii*. Original.

B.—A portion of a wilted shoot, showing the white mycelium and sclerotia of *S. Rolfsii* about its base. Original.



A



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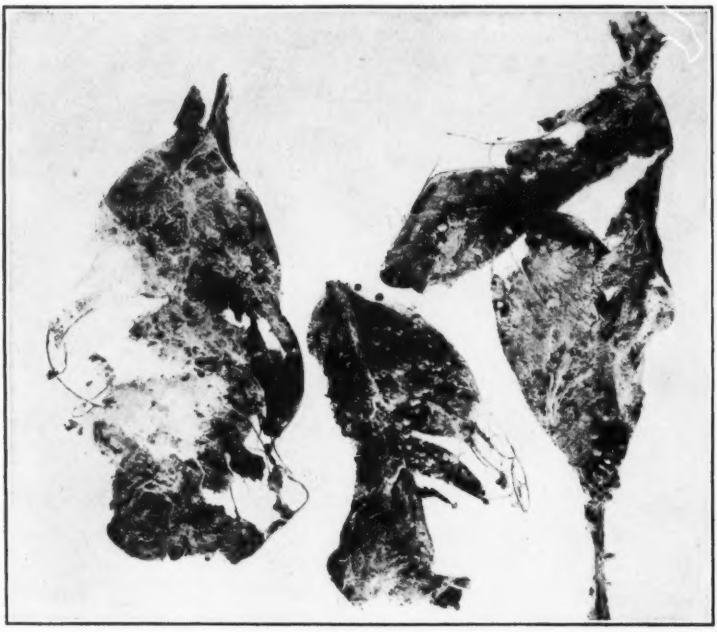

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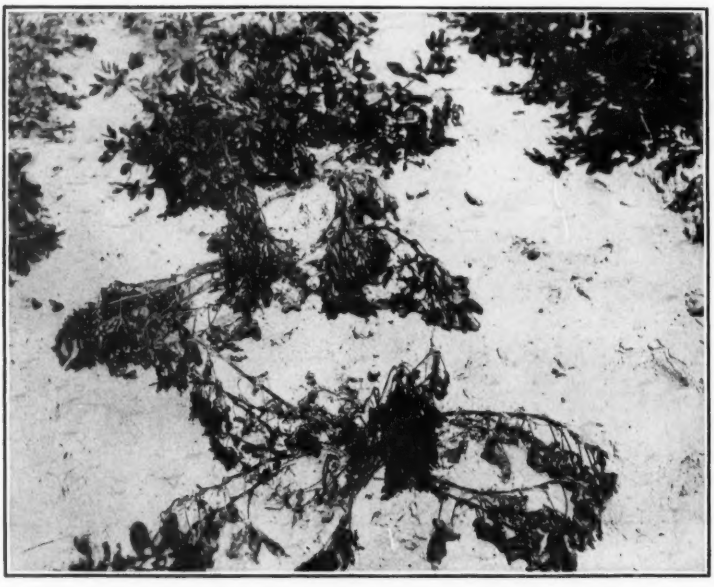

B

PLATE 97

A.—White mycelium and sclerotia of *Sclerotium Rolfsii* on fallen leaflets. Original.

B.—Three hills of peanuts which indicate that *S. Rolfsii* spreads from wilted plants and attacks adjoining healthy plants. Original.

TRANSFORMATION OF PSEUDOGLOBULIN INTO EUGLOBULIN

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INTRODUCTION

In several publications Banzhaf[2] states that when diphtheria serum is heated as it is in the preparation of antitoxin, part of the pseudoglobulin is transformed into euglobulin. Details regarding the methods of analysis or the analytic data on which Banzhaf based his conclusion were not found in the publications examined.

This transformation has both a practical and a theoretical interest. It facilitates the concentration of the antitoxin present in the serum by removing protein without removing any of the antitoxin, so that the final product contains all the antitoxin associated with much less protein. This is desirable because certain of the serum proteins have very little therapeutic value. In applying the heat treatment for the first time to a serum such as anthrax serum, it is obviously desirable to be certain that a similar transformation takes place. Otherwise, there would be no need of the heat treatment, and, besides, the heating may cause loss of potency.

On the theoretical side, the fact that pseudoglobulin can be transformed into euglobulin without affecting the total number of antitoxic units is almost conclusive proof that the antitoxin is a substance separate from pseudoglobulin. That this transformation may take place in some serums, but not in all, is indicated by the following experiments, in which the heating of the serum was carried out under carefully controlled conditions and the analytic data obtained by improved methods. In all, four horse serums were used, as follows:

Anthrax 48.—Serum obtained from horse 48; used in a previous work.[3] Blood drawn January 6, 1916. Potency high.

Anthrax 96.—Serum obtained from horse 96; used previously. Blood drawn January 6, 1916. Potency lower than serum 48.

Diphtheria 1.—A mixture of 500 c. c. of serum, potency 1,400 units per cubic centimeter when bled November 4, 1915, and 700 c. c. of serum, potency 250 units per cubic centimeter when bled October 29, 1915. From two horses.

Tetanus 1.—A mixture of 750 c. c. serum, potency 200 units per cubic centimeter when bled February 14, 1916, and 250 c. c. serum, potency 175 units when bled February 14, 1916. From two horses.

[1] The thanks of the writer are due to Dr. A. Eichhorn, Chief of the Pathological Division, for many valuable suggestions and his interest in the work.

[2] Banzhaf, E. J. The further separation of antitoxin from its associated proteins in horse serum. *In* Proc. Soc. Exp. Biol. and Med., v. 6, no. 1, p. 8-9. 1908.

—— The further separation of antitoxin from its associated proteins in horse serum. No. III. *In* Collect. Stud. Bur. Lab. Dept. Health, city of New York, v. 7, 1912-13, p. 114-116. [1913.]

—— and Famulener, L. W. The proteins and antitoxin in the serum of goats immunized against diphtheria. *In* Collect. Stud. Bur. Lab. Dept. Health, city of New York, v. 8, p. 208-212. [1915.]

[3] Eichhorn, Adolph, Berg, W. N., and Kelser, R. A. Immunity studies on anthrax serum. In Jour. Agr. Research v. 8, no. 2, p. 37-56, 1 fig. 1917.

When received at the Pathological Division these serums were in their native state, without preservative, and had not been filtered. Shortly after their receipt they were Berkefeld filtered and preserved with 0.5 per cent chloroform in a refrigerator.

With the analytical technic described in the previous publication by Eichhorn, Berg, and Kelser,[1] experiments were made on serums anthrax 48 and 96, diphtheria 1, and tetanus 1. The experiments on anthrax 48 and 96 were made in connection with the preparation of globulin for therapeutic use. After filtering the precipitated euglobulin, with or without heating, analyses were made of the filtrates. Obviously the more pseudoglobulin that is converted into euglobulin and precipitated, the less total protein should remain in the filtrate. A large number of analyses obtained on such filtrates, together with those obtained in the experiment in which diphtheria 1 and tetanus 1 were treated with ammonium sulphate, with and without heat, are omitted here because they were inconclusive. In analyses in which 10-c. c. portions of serum or filtrates corresponding to this amount are used, the unavoidable or unknown errors were great enough to obscure the effect of the heat treatment.

HEATING THE SERUM

In the following experiments the serums were heated in a water bath maintained at 61° C. The bath was heated by gas and was provided with a thermoregulator and an electrically driven stirrer. Numerous blank experiments (8) were made in which flasks containing water or one-third saturated ammonium-sulphate solution were heated. One standard thermometer was used for taking the temperature of the water in the bath, while two others were in the flasks being heated. The bath temperature varied from 61° C. by a few tenths of a degree, the bath having been so adjusted that during the experimental heating of the serum the temperature did not rise beyond 61.2° C. nor fall below 60.5° C. This drop was caused by the introduction of the flasks at room temperature, after which the bath temperature rose to almost 61° C. The bath contained about 15 liters of water.

The serum was heated in 200-c. c. Erlenmeyer flasks in quantities of 50 c. c. Two such flasks that were lightest and therefore thinnest were chosen from two dozen. These were always used for heating the serum mixtures. The object was to bring the content up to 60° C., hold it there for exactly 30 minutes, and then rapidly cool it. These two flasks were provided with perforated rubber stoppers, each carrying a thermometer graduated in whole degrees and tested by the Bureau of Standards. The thermometer used in the water bath was tested by the Physikalisch-Technische Reichsanstalt, and was graduated in tenths of a degree. Experiment A was on anthrax 48 and 96, both

[1] Eichhorn, Adolph, Berg, W. N., and Kelser, R. A. Op. cit.

being run at the same time. Experiment B was on diphtheria 1 and tetanus 1, both also run at the same time. On account of the breaking of a centrifuge tube containing a heated diphtheria mixture, the heating was repeated next day in duplicate, so that data were obtained on the heated and unheated single portions of anthrax 48, 96, and tetanus 1, and in duplicate portions of diphtheria 1.

The procedure with a single serum was as follows: Into each of two Erlenmeyer flasks of 200-c. c. capacity, 50-c. c. portions of the serum were pipetted. One of these flasks was always a thin flask used specially for heating. To each flask 25 c. c. of water and 32 c. c. of saturated ammonium-sulphate solution were added, making a total volume of 107 c. c. This procedure was then repeated with the second serum, as it was found convenient to run two together. The two flasks not to be heated were stoppered and set aside. The two to be heated were stoppered with stoppers carrying the thermometers. In the meantime the water bath had been in readiness at 61° C., and the two flasks were introduced. All three thermometers were carefully watched and the temperatures recorded at intervals not exceeding five minutes. The temperatures inside the flasks rose from room temperature, about 27° C., to 56° in the first five minutes of the heating, then to 59° in the next five minutes. After 10 minutes' heating the temperatures inside the flasks were exactly at 60° or below it by only a small fraction of a degree. The heating was then continued for exactly 30 minutes, during which time the temperature inside the flasks did not exceed 60°. It dropped a fraction of a degree for a few minutes on three occasions, but the bath temperature during this time was between 60.7° and 61°, and consequently for all practical purposes the serums may be regarded as having been heated for exactly 30 minutes at exactly 60°, with a preheating lasting 10 minutes. At the end of the heating period the flasks were transferred to a pan containing cold water. This brought the temperature down to that of the room in about five minutes. It is believed that this is as severe heating as is necessary in the preparation of globulin. In preparing large amounts of antitoxin, Banzhaf [1] brings the temperature of the mixtures up to just 60° which required two hours' heating in a water bath kept at 66°.

METHOD OF ANALYSIS

The next step is the separation and estimation of the precipitated euglobulin. In the following scheme of analysis the precipitations of euglobulin and pseudoglobulin are made under exactly the same conditions as in the antitoxin (pseudoglobulin) preparation, and consequently the analytic data may be applied to the corresponding globulin or antitoxin preparations without error. All four mixtures were transferred to 100-c. c. centrifuge tubes, which held about 110 c. c. when

[1] Banzhaf, E. J., 1913. Op. cit., p. 115.

filled to the top. After carefully draining the flasks the small amounts remaining in them were disregarded. The tubes were centrifuged for 25 minutes at about 2,500 revolutions per minute. The sedimentation was perfect and the euglobulin was firmly packed at the bottom of the tubes. The supernatant liquids were poured into 100-c. c. volumetric flasks. They may be poured through filter paper to make certain that no particles are poured off; but this is not necessary, as the euglobulin is sticky and firmly adheres to the bottom of the tube, which may be inverted without loss of precipitate. These 100-c. c. flasks should be weighed when dry and graduated in whole cubic centimeters on the neck, as the volume of liquid poured off may be more or less than 100 c. c. by 2 or 3 c. c. The volumes should be noted to be certain that they are the same for both the heated and unheated mixtures of the same serum.

The saturated ammonium-sulphate solution used was neutral to alizarin sulphonate, and it had been filtered through cotton and hard filter paper. The specific gravities of the saturated, one-half saturated, and one-third saturated aqueous solutions of ammonium sulphate were determined with a Westphal balance and found to be as follows at 26° C.: 1.250, 1.142, and 1.089. These figures were of value in calculating from the weight of the flask and contents the amount of supernatant liquid obtained. The supernatant liquid was used for the estimation of (1) pseudoglobulin, (2) albumin, and (3) these two together, in the form of total coagulable protein.

The precipitated euglobulin in the centrifuge tubes was dissolved in water and transferred to 400-c. c. beakers. These were heated up to the boiling point to coagulate the euglobulin, which separated out in large flocks. The addition of acid was not necessary, although in some instances 1 c. c. of *N*/5 acetic acid was added to favor flocculation. The precipitates were then filtered on weighed papers, washed free from sulphate, washed with small amounts of alcohol and ether, dried to constant weight in the air oven at 100° C., and weighed.[1] In only one instance was there any difficulty in securing flocculation—namely, in a heated diphtheria-euglobulin precipitate. When this occurs, there is, of course, a loss through the passage of unprecipitated protein into the filtrate, and generally the filtrate is very cloudy. That the result would be low in the case referred to (see Tables I and II) was noted before the determination was completed. The heated euglobulin apparently was different from the unheated. The latter dissolved readily in water, forming a clear solution, while the heated euglobulin dissolved much more slowly, forming a milky suspension which became almost water-clear on standing overnight.

Table I contains the results for euglobulin obtained from 50-c. c. portions of serum.

[1] For details see Eichhorn, Adolph, Berg, W. N., and Kelser, R. A. Op. cit.

TABLE I.—*Weights of euglobulin obtained from 50-c. c. portions of serum*

Serum.	Without heat.	With heat.	Increase in weight.
	Gm.	*Gm.*	*Gm.*
Anthrax 48	0.774	1.201	0.427
Anthrax 96	.547	.793	.246
Diphtheria 1	.406	.719	.313
Do	.435	[a].642	.207
Tetanus 1	.243	.304	.061

[a] Result low, probably due to incomplete flocculation.

The figures in Table I show unmistakably that more euglobulin precipitate was obtained from the heated serum than from the unheated. Obviously, it was desirable to ascertain definitely where the excess of euglobulin came from, and to check the correctness of these single results. Concordant duplicates do not prove correctness of results in these analyses; they do prove uniformity of technic and uniformity of error.

In the supernatant liquid (filtrate) poured off the euglobulin precipitates, the following determinations were made: Total coagulable protein (pseudoglobulin plus albumin), pseudoglobulin, and albumin. The method used was as follows:

TOTAL COAGULABLE PROTEIN.—Duplicate portions of the filtrate of 20 c. c. each, containing 9.35 c. c. of serum, were transferred to 400-c. c. beakers; about 300 c. c. of water were added and the mixture brought to a boil. The coagulum, consisting of pseudoglobulin and albumin, flocculated easily. This was filtered on weighed papers as usual, dried, and weighed. The results are tabulated as item C in Table II.

PSEUDOGLOBULIN.—A single portion of 25 c. c. of the filtrate, containing 11.68 c. c. of serum, was used. To this, 10 c. c. of saturated ammonium-sulphate solution were added, resulting in 50 per cent saturation. This was done in a 100-c. c. centrifuge tube and the mixture centrifuged for 20 to 25 minutes at 2,500 revolutions per minute. The pseudoglobulin packed firmly in the bottom of the tube. The supernatant liquid was poured off and its volume noted. This is the solution used for albumin determination. The precipitated pseudoglobulin is dissolved in water, transferred to a 400-c. c. beaker, heated, coagulated, filtered, dried, and weighed as usual. The results are tabulated as item D in Table II.

ALBUMIN.—Of the above fluid from pseudoglobulin precipitation 25 c. c., containing 8.34 c. c. of serum, was diluted and heated, and the coagulated albumin filtered, dried, and weighed as usual. The results are tabulated as item E in Table II.

The figures for euglobulin in Table II, item B, are the same as those in Table I divided by 5. No corrections were made for the volume of the precipitate in any case. The main objects of obtaining these data were, as stated before, to ascertain the source of the excess of euglobulin

in the heated precipitates, and to determine the accuracy of the method of analysis. Theoretically, A should equal the sum of B and C, and C should equal the sum of D and E. Although this equality is absent, the differences appear to be consistent, and indicate a uniformity of error due, possibly, to the absence of corrections for volume of precipitate, solubility of the precipitate in the wash water, occluded ammonium sulphate, etc. These errors, however, do not invalidate a comparison of the data in one column with those of the same serum in the next.

TABLE II.—*Analyses of heated and unheated serums.*

[All weights calculated to grams in 10 c. c. of serum.]

Serum.	Anthrax 48.		Anthrax 96.		Tetanus 1.		Diphtheria 1.			
	Unheated.	Heated.	Unheated.	Heated.	Unheated.	Heated.	Unheated.	Heated.	Unheated.	Heated.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
A. Total coagulable protein....	0.674	0.674	0.789	0.789	0.558	0.558	0.665	0.665	0.665	0.665
Do.....	.618	.618	.788	.788	.....	.....	.....	.....	.....	.....
B. Euglobulin.....	.155	.240	.109	.159	.049	.061	.081	.144	.087	[a].128
C. Pseudoglobulin and albumin estimated together...	.437	.378	.668	.625	.447	.451	.534	.487	.523	.484
D. Pseudoglobulin.....	.290	.211	.568	.535	.385	.378	.475	.397	.479	.438
E. Albumin.....	.191	.191	.133	.135	.103	.103	.090	.098	.087	.097
Pseudoglobulin converted into euglobulin, per cent..	27		6		0.2		16		9	

[a] Result low; see footnote to Table I.

DISCUSSION OF RESULTS

It will be noticed that the figures for albumin are practically identical in the heated and unheated serums. The figures for pseudoglobulin are consistently lower in the heated serums than in the unheated, which is an almost necessary consequence of the transformation of part of the pseudoglobulin into some protein having several of the precipitation characteristics of euglobulin. What is most important for the present investigation is that the loss of pseudoglobulin in the heated serums corresponds almost quantitatively with the gain in euglobulin in the same serums.

Obviously the figures for euglobulin, pseudoglobulin, and albumin are interdependent, and an error in one may cause a corresponding error in another. The figures for total coagulable protein, however, are independent of the others, and an error in them has no direct influence on the figures for the others. The two figures for total coagulable protein in the anthrax serums were obtained January 11, 1916 (upper figures), and May 9, 1916 (lower figures). The comparatively large difference between the two consecutive determinations in anthrax 48 may perhaps be due to the action of serum protease, which continued to digest the serum proteins. Chloroform does not prevent this action when the protease is active. The figures obtained were 0.676, 0.672, and 0.616

and 0.619 gm. The averages of these are the figures recorded in Table II. The corresponding figures for anthrax 96 were 0.756, 0.789, and 0.785 and 0.792. The first figure was rejected, being obviously erroneous. The corresponding figures for the diphtheria and tetanus serums were obtained February 28, 1916. The figures for euglobulin, pseudoglobulin, and albumin were obtained in May, 1916.

The percentage of pseudoglobulin transformed into euglobulin by the heat treatment is calculated by taking the difference between the pseudoglobulin in the unheated and heated serums and dividing by the amount of pseudoglobulin in the unheated serum. These results are tabulated at the bottom of Table II. Thus, for anthrax 48 the figures are $\frac{0.290-0.211}{0.290}$, which equals 27 per cent. However, this is not the only way to calculate this figure. The results for pseudoglobulin may be obtained by subtracting the figures for albumin from those for pseudoglobulin plus albumin. If the percentage of transformation be calculated from the lower values for pseudoglobulin, the figures are 24, 8.4, −1.2, 12.4, and 11.2, respectively, reading across the bottom of Table II. It is expected that further work on the methods of analysis will yield better results.

From Table I it is apparent that the increase in euglobulin is easily determined when a 50-c. c. portion of serum is used. When, however, the filtrate is divided into several portions for the other determinations, the errors in analysis become proportionately large. It is furthermore apparent that in general the amount of pseudoglobulin transformed is not large, considering the long period of heating at a comparatively high temperature—that is, 30 minutes at 60° C. The extremely small amount of the transformation in the tetanus serum leads to the inference that while the transformation may take place when serums are heated, it does not necessarily always take place. This might not be noticed when handling large volumes of mixed serums.

This work was completed several months before the recent investigations of Homer[1] came to the author's attention. Homer states (p. 291) that there is no conversion of pseudoglobulin into euglobulin when serum mixtures were heated to 61° or 63° C. It is probable that the failure to observe the transformation was due to the use of analytical technic that was not delicate enough and to the errors incidental to the handling of large amounts of serum mixtures, as in the routine preparation of antitoxin. Better analytical results can undoubtedly be obtained when using small amounts of serum under conditions adapted to exact analysis and separate from antitoxin preparation.

[1] Homer, Annie. On the concentration of antitoxic sera. *In* Biochem. Jour., v. 10, no. 2, p. 280–307. 1916.

—— An improved method for the concentration of antitoxic sera. *In* Jour. Hyg., v. 15, no. 3, p. 388–400. 1916.

CONCLUSION

The transformation of pseudoglobulin into euglobulin was observed in four serums that had been heated 30 minutes at 60° C. in the presence of 30 per cent saturation ammonium sulphate. In some instances the amounts transformed were considerable, although in one of the serums the amount was so small as to indicate that the transformation does not take place in all serums.

The methods of analysis were improved by the use of the centrifuge as a means of separating globulin precipitates from their filtrates. The precipitations in the analyses were made at the same dilutions as in the precipitations of globulin for therapeutic use.

DISSEMINATION OF THE ANGULAR LEAFSPOT OF COTTON

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INTRODUCTION

A satisfactory explanation of the dissemination of many of the diseases of plants is lacking. The literature recognizes such agents as insects, wind, tools, laborers, drainage, spattering rains, etc., and yet in some cases one must doubt the importance attached to these by investigators. In other instances, particularly in the cases of bacterial diseases affecting the leaves, stems, flowers, and fruits, no satisfactory explanation has been offered. This was the status of affairs in the case of the angular leafspot of cotton (*Gossypium* spp.) when the author began the investigation of this disease at the South Carolina Experiment Station. It is the purpose of this paper to present the data obtained during the past summer and to offer the conclusion reached as to its dissemination under the conditions existing in western South Carolina, with a suggestion of the possible importance of these factors in the dissemination of other similar diseases.

HISTORICAL RÉSUMÉ

A brief résumé of the literature dealing with some of the most common bacterial diseases follows, being presented in order that the true situation may be understood and that the information found there may be used in this discussion.

Beach (1893)[2] and Halsted (1893) concluded that the blight of beans was a bacterial disease, because of the constant association of bacteria with the typical lesions. Halsted further stated that the disease was carried over in the seed. Sackett (1909, p. 21) states that—

> Rain and dew are doubtless agents in spreading the germs from one part of the plant to another by washing them from old lesions onto unaffected parts,

though no evidence of this fact is given. Edgerton and Moreland (1913) successfully inoculated plants without wounding, and concluded that infection can take place through the stomata.

Pierce (1901) successfully inoculated the fruit of the walnut with water suspensions of *Bacterium juglandis*. R. E. and C. O. Smith and

[1] The author is greatly indebted to Prof. R. A. Harper, of Columbia University, for the perusal and criticism of the manuscript, and to Mr. J. W. Sanders, assistant in this laboratory, for his most careful and untiring aid in the work.

[2] Bibliographic references in parenthesis refer to "Literature cited," pp. 473-475.

Ramsey (1912) agree that the uninjured host is susceptible, and conclude that water is apparently the principal agent in conveying the bacteria from the existing lesions to younger leaves and small nuts lower down on the tree. They state (p. 338) that—

> During one of these [before mentioned] fogs the trees became saturated, water dripping from one portion of the tree to another which could easily carry the disease organisms to healthy tissue. Observations go to show that secondary infection in which large numbers of the small nuts become diseased is very likely to follow one of these foggy periods.

Arthur and Bolley (1896) suggest that wound-producing insects are probably an important factor in the spread of the carnation disease caused by *Bacterium dianthi*, though infections can also take place through the stomata. They recommend a method of watering the plants in which the foliage is kept dry, and experience has shown the efficacy of it. In their conclusion (p. 34) the authors state that—

> As there must be moisture upon the leaves sufficient to enable the bacteria to move about and enter the stomata in order that they may gain access to the interior of the leaf, it is evident that keeping the foliage dry will prevent the disease.

It apparently does not occur to them that the water under pressure, being dashed from plant to plant, might serve as a means of dissemination.

Lewis (1914) describes a disease of *Erodium* spp. and *Pelargonium* spp. caused by *Bacterium erodii*, which, he says (p. 230),—

> is more prevalent in crowded beds where the plants remain moist and light is not so dense.

Sprinkling is suggested as the most probable method of dissemination.

Sackett (1910) discusses a disease of the stems of alfalfa caused by *Pseudomonas medicaginis*. The organism is probably carried from place to place on wind-blown dust particles. O'Gara (1914) describes more fully the characteristic appearance of the affected parts, and adds that stomatal infections may occur, though by far the greatest infection takes place through openings produced by insect puncture and severe frost injury.

E. F. Smith, in the second volume of his work on Bacteria in relation to plant disease (1911), summarizes the data relating to water-pore inoculation of cruciferous plants with *Bacterium campestris*, and, in discussing Fischer's objections to this conclusion, writes (p. 308)—

> * * * (2) the hypothetical, dust-dry, wind-borne bacterium requiring a half day or more to moisten it, is probably not the one that usually enters the water-pores and induces the disease, but rather a fresh germ recently come from the interior of some affected leaf as an extrusion from some water-pore already diseased, or left in the vicinity of the water-pore by some wandering insect. * * * such a bacterium would be ready to grow as soon as it found lodgment in a moist place.

The disease progresses most in periods of frequent rains. Russell (1898, p. 31–32), writing on the same subject, states:

> One direct agent by which the disease is spread is the wind. Whether the disease germ is present in the soil or in decaying plants, the dried bacilli can be carried through

the air * * * Inasmuch as this [infection through the water pores] is by far the most common gate of entrance for the disease organism, it is highly probable that the disease is disseminated by means of the wind more than in any other way.

Smith discusses the wilt of cucurbits in the same volume and presents evidence of the dissemination of that disease by insects, especially one *Diabrotica vittata*. Concerning Cobb's disease of sugar cane, this author in his third volume (1914) writes (p. 48):

We can well imagine, however, that under ordinary field conditions, with an abundance of dew or rainfall, and plenty of insect depredators, diseased plants might readily infect neighboring healthy ones, especially when young.

In the chapter on Stewart's disease of sweet corn, which is also in the third volume, the author states (p. 124), in the discussion of an experiment relating to seed infection:

If the disease was actually derived from the seed-corn there probably would have been some cases during the seedling stage, and fragments of these soft plants full of the bacteria would have been blown upon neighboring plants, or dragged by cultivators, or carried on the feet of men and horses, or bitten into by insects, or washed about by rains and dews. There are ways enough to account for the dissemination of the bacteria in the infection of a few plants when the distance is only a matter of a few feet.

In an address before the Massachusetts Horticultural Society (1897) Smith discussed the subject under the headings: (1) Spread by insects; (2) spread by snails and slugs; (3) spread through manure pile; (4) spread by way of the soil; (5) spread by way of seeds, seedlings, buds, tubers, cuttings, and nursery stock.

Macchiati (1891) and Boyer and Lambert (1893) describe a leaf and twig disease of mulberry caused by bacteria. Both claim to have isolated the organism, and the latter authors report successful inoculations with a bacterium named by them "*Bacterium mori.*" E. F. Smith (1910) uses this name[1] for an organism which he determined to be the cause of the same disease in Georgia, though it differed from that of Boyer and Lambert. In no case, however, is an explanation of the method of dissemination offered.

Manns (1909) concluded that the bladeblight of oats was caused by two bacteria to which he assigned the names "*Pseudomonas avenae*" and "*Bacillus avenae,*" and that these bacteria were present in the soil reaching the host through "spattering rains." Manns and Taubenhaus (1913) report their studies of the streak disease of sweet peas and clovers which they found caused by a bacterium named by them "*Bacillus lathyri.*" Later, Manns (1915) discusses this disease and considers more fully the subject of dissemination, saying (p. 12) that the disease attacks the plants about the beginning of the blooming period—

having its origin usually near the ground, indicating distribution by spattering rain and infections through the stomata.

Neither of them states by what means the upper parts of the plants become infected.

[1] According to Migula's system of classification this name would be "*Pseudomonas mori.*"

Griffin (1911), Barss (1913, 1915), and Rees (1915), writing separately of the gummosis of cherry trees, agree that the disease is caused by a motile bacterium, and suggest that sucking insects are the probable agent in its dissemination. Barss (1915, p. 239) says:

> The exact manner in which the disease is disseminated is not known, but indications seem to point to the possibility that sucking insects may be largely responsible for infections.

No data are available in the literature as to the susceptibility of any part of the plant without previous injury, all inoculations having been made by needle puncture.

Stewart and Leonard (1913) show clearly that certain sucking insects, among them the tarnished plant bug, are capable of inoculating tender shoots of pears when *Bacillus amylovorus* is present at the immediate point of puncture or upon the mouth parts of the insect. These authors (1915) conclude that certain flies are not active agents of inoculation, but, because of their abundant presence in the orchards and among nursery stock, they are probably a factor in spreading the bacteria from place to place. Attention is attracted by Stewart (1913, 1915) to the increased disease following rainy weather, due to both the increased susceptibility of the host because of the more succulent growth and the increased activity of the bacteria because of more favorable environment. Heald (1915) is of the opinion that leaf infection of pears by fireblight is possible through water pores, while Hotson (1915, 1916) offers further evidence in support of this.

Rolfs (1915), writing on the disease of stone fruits caused by *Bacterium pruni*, states that infection takes place through stomata and that a film of moisture is necessary to successful inoculation. He says (p. 416, 421):

> Rain and dew are not only important factors for inoculation, but they also carry the bacteria to the healthy leaves, twigs, and fruits, and thus frequently serve as agents of transportation. * * * The warm, slow, continued rains of the summer furnish the best conditions for the rapid spread of the disease. Heavy, driving rains of short duration followed by sunshine and winds are not favorable to its spread, since many of the bacteria are washed to the ground, and the leaves are quickly dried off and the few bacteria that may be spread will be quickly dried and killed.

Rolfs (1915), in his discussion of the angular leafspot of cotton, states (p. 17–18):

> Wet weather, of course, materially aids in the dissemination of the organism. Even if the weather is excessively dry, the dew at night will often furnish sufficient moisture for inoculation. * * * If for any reason the first leaves fail to become inoculated in this way [by contact in presence of dew] the movement of the leaves in the wind especially during a storm, will soon bring them in contact with some of the virus on diseased leaves. * * * The rain carries large numbers of the organism to the new tissue and to the ground under the plants. * * * The soil under the infected plants may thus become an important means of inoculating many of the lower leaves.

Hasse's (1915) conclusion that *Pseudomonas citri* is the cause of Citrus canker has been confirmed by Wolf (1916). The latter states (p. 94), regarding its dissemination:

Definite experimental data are wanting on the agencies by which Citrus canker is spread * * *. It is evident that rain and dew are important factors in carrying the disease to unaffected leaves, twigs, and fruits of trees in which the disease is already present.

Even though Stevens (1914) thought the disease to be due to a fungus, he writes (p. 41):

The disease seems to develop and spread rapidly during rainy weather, but it is more or less retarded during periods of drought or in a dry season.

Brown and Jamieson (1913) present their work on a disease of nasturtium and sugar-beet leaves caused by the same organism, which they named "*Bacterium aptatum.*" While the authors state they had no opportunity to study this disease under field conditions, their experiments would suggest that infection takes place only in bruised or wounded tissue caused by insects or mechanical injury.

A number of preliminary reports of work on bacterial diseases have been made in recent years; and, since in most of these the subject of dissemination is not mentioned, the present author takes it that this phase will be discussed later and, therefore, he will not review this literature.

The facts in the dissemination of certain types of fungus diseases have a close bearing on the subject; yet this literature is so voluminous as to be impossible of review here. However, because of its relation to one of the most serious cotton diseases, anthracnose, Whetzel's (1906) paper on bean diseases is mentioned. Of anthracnose, the author writes (p. 205):

The spores may be scattered by the cultivator, the pickers, by animals, or by the wind in damp or rainy weather.

EXPERIMENTAL INVESTIGATIONS

Infection of the leaves of cotton by *Bacterium malvacearum* can easily be brought about by superficial inoculation in the presence of sufficient moisture. This has been done by applying small amounts of agar-slant cultures of the organism with glass rods and spreading with rubber-gloved fingers when the dew was on the leaves, by applying water suspensions of the bacteria with cotton swabs at all times of the day or evening, spraying such suspensions with an atomizer, or by painting them upon the leaves with camel's-hair brushes. The first signs of infection are minute, dark-green, angular (triangular or quadrilateral) spots on the underside, whether the inoculation was made upon the upper or lower surface of the leaf, usually in 7 to 10 days, though often not earlier than 15 days. In a day or two following the first appearance on the underside of the leaf the same dark-green water-soaked spot will appear on the upper side, though less conspicuously angular. When held up to the light, such spots show a translucency as contrasted with the light, impervious normal leaf and the irregular transparency of some insect injuries. The spot usually increases in size simultaneously on both sides of the leaf, but never crosses the veins. Single infections seldom increase to a size larger than 3 to 4 mm. in the longest dimension. The larger spots, so con-

spicuous on the leaves, are usually caused by the coalescence of two or more separate infections which often occur in clusters when in the mesophyll regions and in a linear direction when along the veins, seemingly where the water gathers and is last to evaporate.

The top side of the spot thus appearing becomes a reddish brown color over a circular area at the center and shrinks slightly in thickness. The colored area increases in size until it conforms to the angular shape of the affected part, the red advancing to the margin and the center shading off to gray. On the underside the spot becomes a brown color, though the dark green, characteristic of the young spot, remains as a narrow band about the margin until the latest stages of development, a place where the activity of the disease continues longest. When the green band finally disappears, the brown replaces it, giving a sharp angular margin to the spot. These color changes may develop quickly, so that the entire spot will be brown eight or nine days after inoculation; but usually they proceed more slowly, leaving the spot dark green for five or six days.

SEED INFECTION

The source of bacteria for the first infections of each season is as yet undetermined, because of conflicting experimental results obtained during the past summer; however, such a problem can be solved satisfactorily only when great numbers are used under the most favorable circumstances. One experiment, or series of experiments, can not be expected to settle the matter.

Over 2,500 seedlings from seed of various sources grown during the winter in all variations of temperature and moisture in the greenhouse and laboratory failed entirely to show the disease. Two instances of cotyledonary infection occurred in the late spring upon seedlings in the greenhouse. An acre field planted on April 20 was entirely free from the disease until August, except in such portion as had been used for inoculation experiments. Another field near by, planted partly with these same seeds and partly with others on May 15, developed considerable disease in both lots of seedlings, though the season was rainy and the chopping was delayed; and no data as to the amount of disease due to the seed and that due to local dissemination could be secured. It was true that, of the diseased seedlings examined, equally as many had cotyledons free from the disease (84) as had them spotted (78).

In order to decide more carefully the probability of seed infection, an experiment was arranged in which samples of various lots of seeds were planted in plots, seven rows wide, the rows being 50 feet long and 3 feet apart. Because of the triangular shape of the land available, these dimensions could not be strictly adhered to; yet each lot of seed was represented by one such plot at least. Most seeds used were samples obtained from several growers in various parts of the State, who had fields badly infected early in the summer, such as would likely be due to

a high percentage of seed infection. These are labeled "Lots II–V," Lot I being seed of the same lot planted on April 20 and remaining free from seedling infection. The other lots (VI–X) are Mississippi Cook seed grown on the Experiment Station farm under differing conditions the previous summer.

On two sides of this triangular plot were cotton variety tests of the Station, and the disease was present upon these plants. To guard against insect activity in spreading the disease to the young seedlings and thus confusing the results, cages of cheesecloth were erected over 12 feet of a row of each lot of seed, giving an opportunity for the disease to appear under such protection if the bacteria were present upon or in the seed. The field was planted on August 12. The seed germinated quickly, and the seedlings began to appear above the ground by the 16th and 17th. On the 22d a careful examination was made of all the plants, but no disease was found. On the 28th 11 cases of cotyledonary infection were found, and on September 1, 20 days after planting and 15 days after the cotyledons were spread, affording ample time for the appearance of the disease in view of our earlier work, the final counts were made. Of 34 diseased plants found, only one was beneath a cage; yet that one lends weight to the conclusion that seed infection does occur. The tabulated data by plots of each lot of seed follows in Table I.

TABLE I.—*Results of the infection of cottonseed by angular leafspot*

Lot No.	Plot No.	Number of seedlings.	Number diseased in—		Total number of seedlings.	Total number diseased.	Percentage diseased.
			11 days.	20 days.			
I	1	820	0	0			
	2	4,926	0	1			
	3	1,743	0	0			
	4	586	0	0			
	5	468	0	0			
	Cage.	207	.....	0	8,750	1	0.011
II	6	3,023	4	2			
	7	3,543	0	7			
	Cage.	179	.....	1	6,745	14	.2
III	8	2,622	4	1			
	9	700	0	0			
	Cage.	86	.....	0	3,408	5	.14
IV	10	2,884	0	6			
	11	1,242	0	4			
	12	1,402	0	8			
	Cage.	117	.....	0	5,645	18	.31
V	13	142	0	0	142	.....	.....
VI	14	2,030	0	0	2,030	.....	.....
VII	15	1,150	3	4			
	16	195	0	0			
	Cage.	145	.....	0	1,490	7	.46
VIII	17	2,002	0	0			
	18	1,247	0	0	3,249	0	.....
IX	19	3,678	0	0	3,678	0	.....
X	20	1,415	0	0			
	Cage.	16	.....	0	1,431	0	.....
Grand total					36,568	45	.....

In this experiment the seedlings of Lot I were slightly diseased (0.011 per cent), the results being comparable with those obtained from the field planted on April 20. It is improbable that the diseased plants in the field planted with these seed on May 15, which varied from 2.7 to 8.1 per cent of the plants of single rows, were due to seed infection, but rather to the spread of the disease from other infected plants at an extremely favorable time.

The other results obtained, 0.2, 0.14, and 0.31 per cent, do not account for the amount of disease observed in fields planted with these seed. The field planted with seed of Lot V was the most badly diseased of any seen on a trip about the State in June; and, while the author obtained only 142 seedlings, none of them were diseased.

Without doubt, 0.46 per cent of diseased seedlings at the beginning of a season, especially if rainy weather prevails before chopping, would be sufficient to start a general field infection, and this could be called the primary infection. It is interesting to note in this connection, however, that 1,218 seedlings grown in the greenhouse from seeds planted on August 5 and taken from the same bag as those in Lot VII were entirely free from the disease as late as August 29. It is improbable that the conditions in the greenhouse were unfavorable to the development of the disease, since successful artificial inoculations have been made here besides the two cases of natural cotyledonary infection already mentioned; and, if we accept this as the case, adding these seedlings to those observed in the field, making a total of 7 diseased seedlings in 2,708, the 0.46 per cent is reduced to 0.25 per cent.

Whatever the true situation in this regard may be, and only further carefully checked observations can decide, it is a fact that the disease appears sooner in some parts and later in others, yet almost inevitably in every cotton field. In any method of primary infection it is improbable that every plant will be attacked, so that the spread of this disease from leaf to leaf and plant to plant becomes a subject of considerable interest and importance.

INSECT DISSEMINATION

In view of all the work—much of it recent—done on the subject of the spread of plant diseases by insects, an effort was made to determine carefully the extent of their activities in the dissemination of the angular leafspot. Leaf-eating beetles (flea beetles and cucumber beetles) were abundant in some fields of seedling cotton, but in only one case has the author observed the disease developing about the margin of eaten areas. The most common insects upon the older plants were the jassids; and, since they were more active than any other except the ants (the latter, however, being active only after the dew had disappeared), especial attention was given them. Five large plants were

caged in the greenhouse, with cheesecloth stretched over wooden frames. Into one of these cages were placed 50 jassids caught in the early morning while the dew was on the plants; into another, 75 jassids caught about 9 a. m., after the dew had evaporated. Two cages were used as uninoculated controls, while the plant in the fifth cage was sprayed with a water suspension of a 6-day-old 1 per cent saccharose agar slant culture of *Bacterium malvacearum*. After the insects had been upon the plants for 18 days in one instance and 14 days in the other and after the inoculated control had developed for 11 days the cages were opened and observations made. The results are given in Table II.

TABLE II.—*Results of a greenhouse experiment to determine the agency of insects in the dissemination of angular leafspot*

Test.	Number of leaves not infected.	Number of leaves infected.
Jassids caught in dew (18 days)	58	1 (1 spot?).
Control	58	0
Jassids caught dry (14 days)	132	0
Control	124	0
Inoculated control	99	22 (871 spots).

While the data are not conclusive as to the activities of these insects the conclusion that they have a very slight effect, if any at all, is supported by the later developments in the same patch used for seed-infection studies and described above.

Shortly after September 1 this field was chopped to a close stand and the plants allowed to develop. There was considerable angular leafspot present upon the plants in the adjoining fields, and an excellent opportunity was presented for the insects to demonstrate their influence upon the spread of the disease, since the growing plants were at a favorably susceptible age. A careful examination was made of this field on October 2, particularly of those plots lying next to the older cotton. Table III shows the amount of disease present, the data being presented in rows per plot. Those of the first five plots were parallel to the rows of the adjacent cotton field, the seventh row being slightly over 21 feet away, while the remaining plots were bordering the cotton on another side of the triangle, with the rows running toward the other field, so that no idea of distance can be had. The numbers of the plots are identical with those in Table II, which facilitates comparison.

TABLE III.—*Results of a field experiment to determine the agency of insects in the dissemination of angular leafspot*

Row.	Plot 1.		Plot 6.		Plot 14.		Plot 8.		Plot 2.		Plot 10.		Plot 9.		Plot 4.		Plot 5.		Plot 12.		Plot 18.	
	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.	Plants.	Plants diseased.
1	41	1	49	0	35	0	46	1	80	0	51	4	25	0	19	0	69	0	52	1	46	0
2	35	0	50	0	41	0	38	1	78	0	54	2	26	0	21	0	64	0	47	0	31	0
3	31	0	53	0	40	0	56	0	83	0	45	1	20	0	20	0	.....	.....	41	0	32	0
4	21	0	48	3	38	0	49	0	75	0	48	0	23	0	16	0	.....	.....	37	0	29	0
5	21	0	61	0	37	0	53	0	65	0	42	0	18	0	14	0	.....	.....	.....	.....	17	0
6	9	0	65	2	44	0	43	0	58	0	60	0	19	0	16	0	.....	.....	.....	.....	15	0
7	6	1	52	0	44	2	44	0	47	0	36	2	0	0	11	0	.....	.....	.....	.....	.....	.....
Total	154	2	378	5	279	2	329	2	486	0	336	9	131	0	97	0	133	0	177	1	170	0

Considering the irregular distribution of the diseased plants in plots 1, 6, 14, 8, and 2, little evidence of insect activity is noted; and this fact, together with the higher percentage of diseased plants in plot 9, which was credited with high seedling infection (Table I), might lead one to believe this disease present to be more likely due to seed infection or spread from such plants.

DISSEMINATION BY WIND DURING RAINFALL

An extensive inoculation experiment was arranged in one of the college cotton fields to demonstrate beyond all doubt the pathogenicity of the organism which preliminary inoculations indicated was *Bacterium malvacearum*. This field was located on a plateau overlooking a valley 2 miles wide sloping from east to west, with no higher hills near by. The row chosen for the experiment was parallel to a roadway and about 20 rows from it (80 feet). The inoculations were made on May 26, one person applying the bacteria from agar-slant cultures to the underside of two or three leaves of each plant by means of a sterile glass rod. One tube culture and one rod were used as far as the culture served, when another culture and rod were used. The bacteria were gently spread over the underside of the leaf with the fingers by a second person, using rubber gloves to facilitate sterilization after each culture. The success of the experiment was noted on June 3 (eight days later), though a complete record was not taken until June 5, when an examination showed that none of the control plants were diseased and that all those inoculated were diseased except two. These also showed infection shortly afterwards.

A record was taken on June 5 of the number of leaves infected and uninfected. Comparison with a similar record taken on June 15 showed an increase from 2 to 10 leaves per plant, usually 3 to 6, and a similar

B

142 146 148 143

143 151 140 145 Chk.

Chk.

153

Chk.

| | | |

A

FIG. 1.—Diagram of the exp
of the disease per plant
Second row; same on Ju

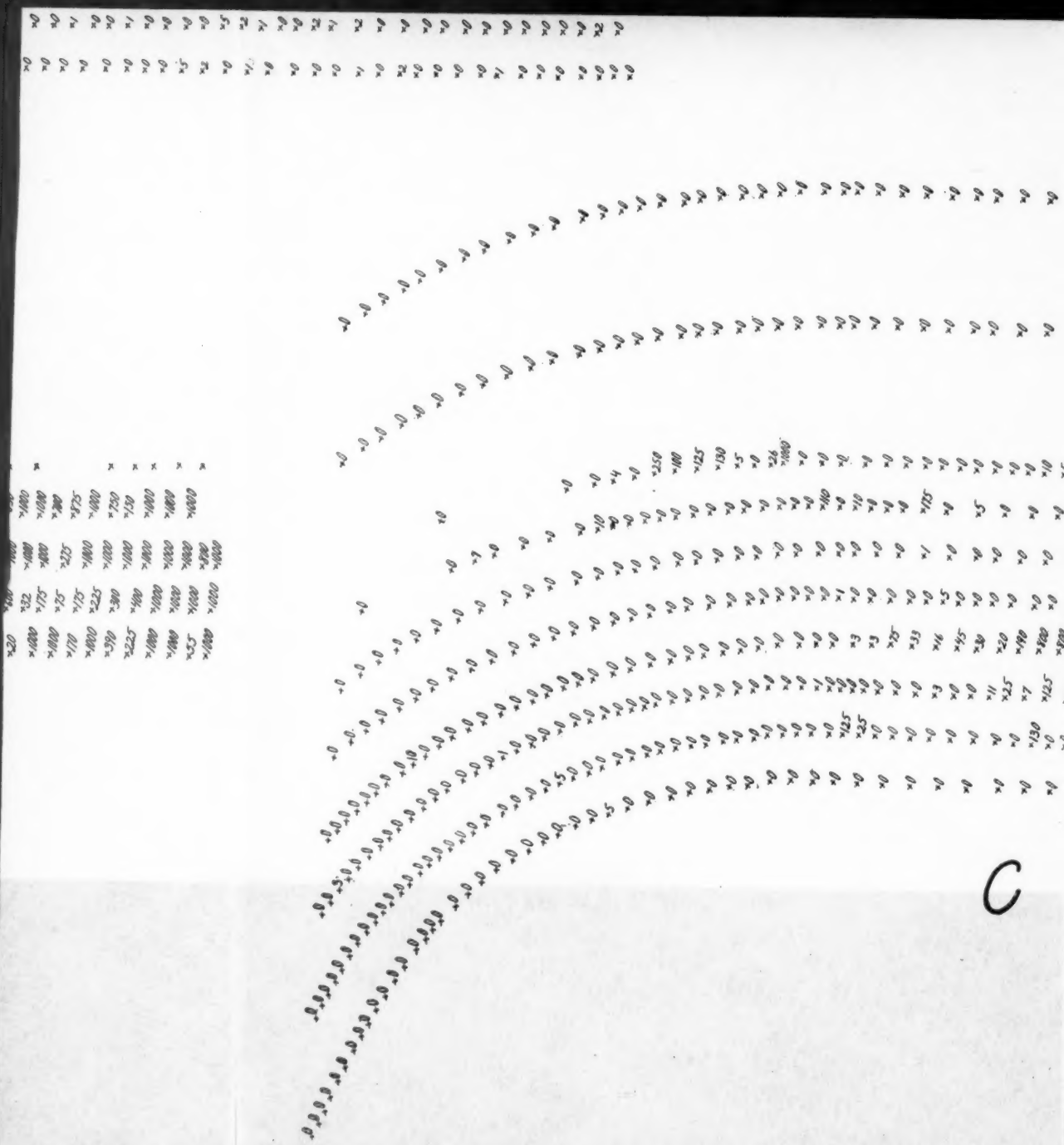

FIG. 1.—Diagram of the experimental field of cotton: *A*, Part of the plants diagramed in *B*, showing the reversal of spread between June 27 and July 30. Numbers of 1,000 are relativ
of the disease per plant on June 27. Numbers below the inoculated plants refer to the culture number of organism used. Note the spread of the disease over the checks in the same
Second row; same on July 15, showing the slight infection. Below: Chart of neighboring plants, showing spread of the disease on July 30 to the west and the relative freedom of the r

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1,000 are relative, denoting 1,000 or more. *B*, Portion of the field in which the row inoculated on May 26, 1916, was located. Figures show spots
ks in the same row—also that toward the east for 14 rows and toward the west 1 row. *C*, Top row: Row selected for inoculation on June 30.
freedom of the rows to the east.

increase of diseased leaves. At the time two control plants standing next to inoculated plants were also diseased. Thus, there is without doubt a rapid increase of the number of the spots on infected plants and those adjacent, probably due, as Rolfs (1915, a) points out, to the spread of the bacteria by contact with uninfected leaves and plants in the presence of sufficient moisture.

During the observations made on June 26, to note the spread of the disease in this field, it was found that the row on each side of that inoculated contained infected plants, though the disease appeared to be spread farther toward the east than the west. That part of the field was carefully diagramed, an accurate record made of the disease on each plant, and the data then charted for closer study. The chart is reproduced here to show the true situation in the field at that time (fig. 1, *B*.)

All the plants are represented by cross marks, those originally inoculated with *Bacterium malvacearum* are divided into series, each series being given the number of our culture used. The numbers near the plants of the neighboring rows and of the checks designate the number of angular leafspots found on them on June 27 and 28. The inoculated plants were so badly diseased as to make such data useless.

Two facts stand out upon examination of the chart: (1) There had been very little spread of the disease in a westerly direction as contrasted with that toward the east, and (2) the spread toward the east was strikingly opposite the inoculated plants as compared to the control plants. Upon closer study of these points it will be noticed that one had to proceed 14 rows to the east to find a row comparable in amount of disease with the second one west of the inoculated row. Further, a decrease in disease is noticed west of wide skips in the inoculated row almost as pronounced as that west of the control plants.

The significance of these data was much of a surprise to the author; therefore, he immediately set about to duplicate the original conditions, hoping at least to obtain some partial repetition of these results. A row in the same field, 35 rows west of the first inoculated row, was chosen for the second experiment. For this inoculation a bacterial suspension was made by grinding a large number of badly spotted leaves in a food chopper and diluting the macerated tissue with water. The rapid spread of the disease after the first inoculation led the author to believe that the bacteria in culture had been more or less attenuated, since it was eight days after inoculation before the disease appeared on the leaves. Unless a great amount of this spread had taken place at one time, it would have been difficult to understand how the disease could progress so far in one direction if each spot took 8 to 10 days to appear.

In the row used, a number of plants serving as controls were not inoculated, a number were inoculated, and others again left as controls. A shallow dish of the fresh suspension of bacteria was held in one hand while each of the leaves of the plants to be inoculated was immersed;

in this way inoculations were made with bacteria which had never been in culture. A record made of the amount of disease in this row at the time of inoculation is shown in figure 1, *c*. There were some infected plants, but not enough to interfere with the work as long as their location was known. Frequent examinations of the plants were made; and any idea of attenuation of the virility of the organism by artificial culturing proved unfounded, since few of the spots appeared in less than eight days and most of them later. A record taken on July 15 (fig. 1, *c*) shows how slight the inoculation was, probably because of the high dilution of the bacterial suspension. No count was made, and no idea is now had of the number of bacteria per cubic centimeter of that suspension.

It was fortunate, from the author's viewpoint, that this experiment was arranged before the prolonged rainy period of July, in which rain fell on July 7 and almost every day afterwards until the 25th. During this time the effect of the two tropical hurricanes, which did considerable damage in the Gulf States, was felt, the wind blowing during all the time from the southeast.

Observations made toward the end of July, allowing a time factor as long as we had in our first experiment, showed that the disease had spread to the northwest of the inoculated row with very little disease on the row to the east (fig. 1, c). Upon further examination of the plants in the first experiment the disease was found to have spread over the plants in the rows west of that inoculated (fig. 1, *A*), the direction of spread having been reversed. Many of these plants were recorded as being free from disease on June 28, while on July 30 it was so abundant as to make careful counting needless. Consequently "1,000" is used to denote relatively a great amount of disease, more than 1,000 spots. The only obvious factor which is capable of such far-reaching action is the wind, and this only when sufficient moisture is present over the leaves to enable the bacteria to become detached from the colony within the diseased area, either through the stomata or from the surface of the spots. Even heavy dews afford sufficient moisture for this purpose, as has been demonstrated by experiment.

Absorbent cotton, sterilized in a plugged Erlenmeyer flask, was carried to the field on two occasions in the early morning while the dew was present. Small bits of this were pulled off with forceps, and each bit placed upon the top side of the diseased leaves, not especially over lesions, but promiscuously upon the leaf. After these were placed, each was taken up and placed into a tube containing sterile water (a sterile water "blank"). These were taken to the laboratory, shaken thoroughly, and samples of the water plated in agar. In the first preliminary experiment 5 out of 30 plates showed *Bacterium malvacearum*. In the second case, 12 out of 84 plates showed the organism. It would seem that the close percentages (16 and 14) of these two experiments is merely coincidental;

yet in another experiment in which cold, poured agar plates were taken to the field and with covers removed, dew dashed onto the agar surface, about the same percentage gave growths of *Bact. malvacearum*.

It is reasonable to expect that the bacteria would become as free in a film of water due to rains as to dew. When bacteria have thus escaped from the interior of diseased areas through stomata (possibly in other ways) and have been suspended in this water film, any agency carrying this water from plant to plant becomes a means of dissemination. Wind during rainfall is the most probable agency which has been active in this disease of cotton. Infection by contact of plants is precluded by the distance between rows (4 feet) and the height of the plants at the time of the first experiment—8 to 10 inches at its beginning and not over 18 inches high at its close.

METEOROLOGICAL CONDITIONS

Little can be said concerning the wind during the rainfall which spread the disease in these experiments. Future studies are planned in which these factors will be more closely observed, and it is hoped to learn what variations and what minimum amounts of wind and rain, separately and together, will have this effect. It is a fact of record that the wind blows during the rainfall of thunderstorms of this region, and it is common knowledge that the wind blows violently during the rainfall of West Indian hurricanes. As types of the thunderstorms most important in this regard, three graphs are presented (fig. 2), records of storms observed at the office of the United States Weather Bureau, Little Rock, Ark., and Vicksburg, Miss. The high wind preceding the rain, or at its beginning, should be noted. It is improbable that this wind at the beginning is instrumental in spreading bacterial diseases, but it will be noted that later the wind reaches a velocity of 25, 29, and 35 miles per hour during periods in the storms when rain is falling heavily and after the foliage has been wet for some time. It is extremely probable that winds of these velocities blowing during heavy rainfall will serve to disseminate such diseases as the angular leafspot of cotton.

The records of storms in the extreme western portion of the cotton belt are used here so that it will be noted that these meteorological conditions are not peculiar to this locality but that such conditions are prevalent throughout the cotton-growing States and probably elsewhere as well.[1]

DISCUSSION OF AGENCY

Suitable conditions of wind and sufficient moisture occur during the summer rains of this region. The method of action of this combination has been and will be the subject of close attention. During June, 1916,

[1] Thanks are due to Messrs. H. S. Cole and William E. Barron, officers in charge of the Weather Bureau offices at Little Rock and Vicksburg, respectively, for their kind assistance and suggestions in this phase of the subject.

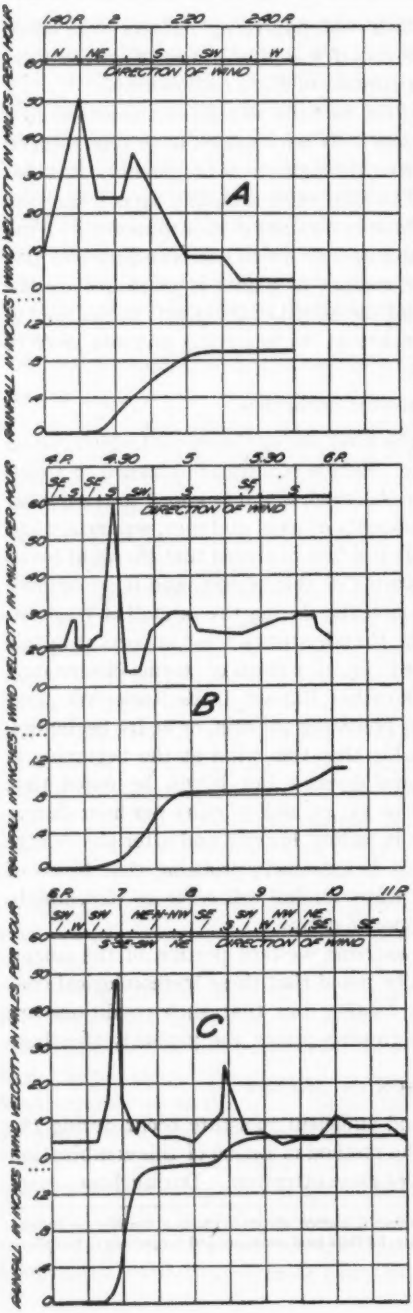
1.40 P. 2 2.20 2.40 P.
N NE E S SW W
DIRECTION OF WIND
A
RAINFALL IN INCHES | WIND VELOCITY IN MILES PER HOUR
4 P. 4.30 5 5.30 6 P.
SE SE SW
DIRECTION OF WIND
B
RAINFALL IN INCHES | WIND VELOCITY IN MILES PER HOUR
6 P. 7 8 9 10 11 P.
SW SW NE NW SE SW NW NE SE
S SE SW NE
DIRECTION OF WIND
C
RAINFALL IN INCHES | WIND VELOCITY IN MILES PER HOUR

FIG. 2.—Graphs showing direction of wind and its velocity and the amount of rainfall during thunderstorms: *A*, Little Rock, Ark., August 22, 1916; *B*, Little Rock, Ark., June 5, 1916; *C*, Vicksburg, Miss., September 25, 1916. Note the high wind velocity during the rainfall in the latter part of the storms.

there occurred one or two typical thunderstorms. During July a rainy period continued through the greater part of the month, though from figure 1 it can be seen that the inoculated plants in the second experiment were only slightly diseased on July 15, most of the spread probably occurring after that time. During this time, however, the effects of two West Indian hurricanes were felt, and the wind blew during most of the rain.

Just how far a falling raindrop could splash water from the surface film of a leaf is hard to conjecture, but this must be a factor in the problem. It is probably this that previous authors had in mind in discussing disease dissemination; yet the distance which the wind blowing at the time of the splash will carry this ascending water is a problem for determination. The possibilities of this chain of action during a driving rain are considerable if one includes the distance bacteria may be carried from the original lesion, then splashed up again and carried farther, and so on, until a dilution too great for infection is obtained.

Rolfs concluded that rains followed by sunshine and wind were not favorable to the spread of the bac-

terial disease of peaches, etc., since the desiccation resulting killed the bacteria which had been spread. The physiology involved in our subject has not as yet been determined—whether negative heliotropism, positive chemotropism, or mere chance determines the time within which the bacteria enter the stomata—but it is true that infection resulted almost uniformly when the inoculation was made in direct sunshine on the upper or lower surface of the leaves with a temporary unprotected layer of water over the leaf surface. It may have been that a small percentage of these organisms withstood the unfavorable environment until night and that penetration of the leaf occurred then. The result of the author's experiments on the effect of sunlight upon *Bacterium malvacearum* agree with those of Rolfs (1915, a) and Edgerton (1912), in that the present writer found that 15 minutes' exposure kills some bacteria, 30 minutes a considerable number, and in an hour almost total destruction of the bacteria in the exposed parts of poured agar plates occurs. Some growth always appeared at the edge of the exposed area, and this was thought to be due to spreading from the margin of the shaded portion. More evidence on this point is needed. The conditions following rains seem to exert little influence on the spread of the angular leafspot of cotton.

Stewart and Leonard (1915, 1916) show conclusively that sucking insects are capable of inoculating young pear trees with *Bacillus amylovorus* when the bacteria are present upon the twigs at the immediate point of puncture or upon the mouth parts of the insects. Flies, etc., also have been determined to be a probable means of spread of the bacteria. To what extent these agents work together, in what other ways the bacteria may be spread from place to place to be inoculated by sucking insects, or to what extent inoculation may follow after insect punctures (since insect activity is lessened by rains, and the spread of the disease, according to these authors, is most noticeable after rainy periods) have not been determined. These authors state (1915, p. 121) that—

> On the other hand, it is to be noted that sucking bugs may be present in great numbers without the occurrence of much blight,

then describe two situations differing only in the amount of rainfall, and conclude that the resistant condition of the trees in the case where the drought prevailed lessened the blight as compared to a severe outbreak in the case of an abundant rainfall.

The work on Citrus canker is of particular interest and importance in this connection because the southeastern Citrus region is subjected to the tropical hurricanes, storms in which the wind blows highest and longest during rainfall and which above all others would serve for this purpose. Hasse determined the pathogenicity of her organism without wounds or injuries of the host plant, and Wolf concludes that rain and dew must serve as agents of local dissemination. If the action of a hurricane be combined with the motility of such a bacterium, in view of

the writer's results, this disease would undoubtedly be spread rapidly, as is noted by Stevens (1914).

The application of these conclusions to other diseases of such plants as walnut, cabbage, curcubits, oats, clover, sugar cane, and sweet corn, caused by motile bacteria which infect the aerial portions of the host, depends solely upon the prevalence of these meteorological conditions—that is, sufficient wind during rain to blow the water from plant to plant. It need not be from host to host, because other plants may serve as "stepping stones," as it were, provided the bacteria reached their host before the suspension became too dilute for infection. Their application is not at all limited to bacterial diseases, since there are many fungi, parasitic upon plants, having spores in sori, such as the Glomerellas and rusts, others having spores in pycnidia and in asci, where water is a necessary factor in their dissemination, and others having aerial spores, such as the Monilias and downy-mildews, where water is not necessary, yet in all probability would greatly augment infection by spreading the spores in the most favorable environment.

It is this factor which places all of these diseases in one theoretical class as regards control. Fungicides have not been generally accepted as a logical means of preventing bacterial diseases, though their efficiency should be the same as in the cases of potato-mildew, apple-scab, etc. A protective compound applied to the susceptible parts, active in the presence of water and of sufficient toxicity to kill the several bacteria quickly, would be expected to have the same result as in cases where sprays are recommended. In fact, Rolfs (1915, a) reports successful results with Bordeaux mixture in the prevention of this disease. Stewart (1913, a) mentions certain unpublished observations by Reddick in which a spray on the blossoms of pear lessened fireblight. Wolf and Massey (1914, p. 100), referring to Citrus canker, state:

> Very encouraging indications of successful control have been obtained by the use of each of these fungicides [Bordeaux mixture, ammoniacal copper carbonate, and soluble sulphur].

Sprays have been tried by some one or another in the case of almost every bacterial disease, and in the majority of instances negative results have been reported. This has developed the more or less current opinion that the theory of such methods is incorrect; whereas from these data one might conclude that the theory is correct, improper fungicides or ill-timed applications having been the cause of failures.

CONCLUSIONS

In conclusion and by way of summary the following points may be reiterated as of greatest importance:

(1) The methods of dissemination of many plant diseases, especially those caused by bacteria, are not satisfactorily described in the literature.

(2) In the case of the angular leafspot of cotton there is evidence of but little seed dissemination, though this is a probable factor in primary infection.

(3) Insects play a very unimportant part in the spread of this disease.

(4) Data have been obtained which point to the conclusion that wind-blown rain is an important factor.

(5) The records of the United States Weather Bureau show that such an agency is possible.

(6) Nothing in the literature precludes the assumption that such an agency may be effective in the dissemination of other similar diseases, while certain facts are on record which render it probable that this factor is of importance.

(7) The control measures used in the case of diseases disseminated by the wind which require water for infection may be expected to serve satisfactorily for this type of disease.

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INDEX

Page

Absorption, arsenical injury of fruit-tree roots by..... 311–313
Acid, arsenic, injury to fruit trees..... 298–299
Acidity, development in fermenting corn silage..... 368–374, 376–377
Albumin, estimation..... 451–455
Alcohol, formation in fermenting corn silage..... 368–373, 376–377
Alternaria sp., temperature relations..... 139–164
Althea. See *Hibiscus syriacus*.
Amino nitrogen, change in fermenting corn silage..... 368–369, 372, 375
Ammonia nitrogen, change in fermenting corn silage..... 372
Ammonium arsenate, injury to fruit trees... 298–299
Anthrax—
  serum. See Serum, anthrax.
  treatment in man..... 52–53
Antigen, tuberculosis—
  comparative tests..... 14–16
  preparation..... 5–10
  standardization..... 8–10
Apiary, spore-forming bacteria—
  comparison of species..... 399–412
  description of species..... 399–412
  inoculation..... 412–420
Apple-rot fungi, temperature relations..... 139–164
Apple. See *Malus sylvestris*.
Arachis hypogaea, wilt of—
  casual organism..... 441–448
  description..... 442–443
  dissemination of..... 444–445
  effect of rotation..... 445
  history..... 441–442
  injury by..... 443
  varietal resistance to wilt..... 445–447
Arsenic—
  acid, injury to fruit trees..... 298–299
  injury to fruit trees..... 283–318
  trioxid, injury to fruit trees.. 299–301, 308, 309, 313
  trisulphid, injury to fruit trees..... 299–301
  washing, effect on fruit-tree injury..... 302–303
Arsenical Injury Through the Bark of Fruit Trees (paper)..... 283–318
Aspergillus niger, temperature relations..... 152
Availability of Potash in Certain Orthoclase-Bearing Soils as Affected by Lime or Gypsum (paper)..... 21–28

Bacillus—
  alvei..... 412–414
  atrosepticus—
    biochemical characters..... 112–119, 123–124
    cultural characters..... 105–112, 123
    description..... 97–98, 122–124
    group number..... 120
    inoculation..... 98–124

Page

Bacillus—Continued.
  atrosepticus—continued.
    morphology..... 103–105, 122–123
    nomenclature..... 121–122
    pathogenicity..... 119–120, 124
    physical characters..... 112–119, 123–124
    relation of temperature to growth..... 116–119
  larvae..... 412, 416
  melanogenes—
    biochemical characters..... 112–119
    cultural characters..... 105–112
    description..... 98
    group number..... 120
    inoculation..... 98–124
    morphology..... 102–105
    nomenclature..... 121–122
    pathogenicity..... 119–120
    physical characters..... 112–119
    relation of temperature to growth..... 116–119
  mesentericus—
    description..... 405–407
    inoculation..... 412–418
  orpheus, description..... 410–411
  pluton..... 412, 417
  phytophthorus—
    biochemical characters..... 112–119
    cultural characters..... 105–112
    description..... 95–96
    group numbers..... 120
    inoculation..... 98–124
    morphology..... 102–105
    nomenclature..... 121–122
    pathogenicity..... 120
    physical characters..... 112–119
    relation of temperature to growth..... 116–119
  solanisaprus—
    biochemical characters..... 112–119
    cultural characters..... 105–112
    description..... 96–97
    group number..... 120
    inoculation..... 98–124
    morphology..... 102–105
    nomenclature..... 121–122
    pathogenicity..... 119–120
    physical characters..... 112–119
    relation of temperature to growth..... 116–119
  vulgatus—
    description..... 407–410
    inoculation..... 412–418
Bacterium—
  malvacearum—
    causal organism of angular leafspot of cotton..... 461–475
    dissemination..... 464–472
    infection of seed of *Gossypium* spp..... 462–464
  tumefaciens—
    causal organism of crowngall..... 165–186
    growth..... 407–409

Page

Bark, fruit trees, spraying injury..... 283–318
Bean, soy. See *Soja max*.
Berg, W. N. (paper): Transformation of Pseudoglobulin into Euglobulin..... 449–456
Berg, W. N., Kelser, R. A., and Eichhorn, A. (paper): Immunity Studies on Anthrax Serum..... 37–56
Bioletti, F. T., and Bonnet, L. (paper): Little-Leaf of the Vine..... 381–398
Blackleg of *Solanum tuberosum*—
causal organisms of..... 79–124
control..... 91–94
description..... 82–85
dissemination..... 90–91
distribution..... 85–87
economic importance..... 87–89
source of infection..... 90–91
Blood, tuberculin tests..... 11–14
Blumberg, A., and Eichhorn, A. (paper): Diagnosis of Tuberculosis by Complement Fixation, with Special Reference to Bovine Tuberculosis..... 1–20
Bonnet, L., and Bioletti, F. T. (paper): Little-Leaf of the Vine..... 381–398
Botrytis cinerea, temperature relations..... 139–164
Bouyoucos, C. J. (paper): Measurement of the Inactive, or Unfree, Moisture in the Soil by Means of the Dilatometer Method..... 195–217
Bovine serum. See Serum, bovine.
Breazeale, J. F., and Briggs, L. J. (paper): Availability of Potash in Certain Orthoclase-Bearing Soils as Affected by Lime or Gypsum..... 21–28
Briggs, L. J., and Breazeale, J. F. (paper): Availability of Potash in Certain Orthoclase-Bearing Soils as Affected by Lime or Gypsum..... 21–28
Brooks, C., and Cooley, J. S. (paper): Temperature Relations of Apple-Rot Fungi.. 139–164
Bruchophagus funebris, host insect of *Tetrastichus bruchophagi*..... 277–282
Butt, N. I., and Harris, F. S. (paper): Effect of Irrigation Water and Manure on the Nitrates and Total Soluble Salts of the Soil..... 333–359

Calcium—
arsenite, injury to fruit trees..... 298–303, 307
hydrate, effect on potassium in—
orthoclase..... 24
pegmatite..... 22–23
soil..... 25
sulphate, effect on potassium in—
orthoclase..... 24, 27
pegmatite..... 22–23
soil..... 25–27
wheat..... 27
Calcium Compounds in Soils (paper)..... 57–77
Carbon dioxid, evolution in fermenting silage..... 373–374
Cephalothecium roseum, temperature relations in apple..... 139–164
Choanephora cucurbitarum—
causal organism of squash disease..... 319–328
chlamydospores..... 324
conidia..... 322–323
description..... 326

Page

Choanephora cucurbitarum—Continued.
development..... 322–326
dissemination..... 326–327
distribution..... 319–320
etiology..... 321
history..... 319–320
parasite of—
Cucumis sativus..... 320, 326
Cucurbita pepo..... 320, 326
Hibiscus—
coccineus..... 320, 326
esculentus..... 320, 326
syriacus..... 320, 326
pathological anatomy..... 326
sporangia..... 323–324
sporangiospores..... 324
zygosporic stage..... 324–325
Citrus aurantium—
effect of fertilizers on—
composition..... 127–138
quality..... 127–138
phosphoric-acid content..... 137
potash content..... 137
effect of soil on composition..... 135–136
Colley, R. H. (paper): Discovery of Internal Telia Produced by a Species of Cronartium..... 329–332
Complement fixation, diagnosis of tuberculosis by..... 1–20
Cooley, J. S., and Brooks, C. (paper): Temperature Relations of Apple-Rot Fungi. 139–164
Copper aceto-arsenite, injury to fruit trees.. 299–309
Corn. See *Zea mays*.
Cotton. See *Gossypium* spp.
Crabapple. See *Malus* spp.
Crimson clover. See *Trifolium incarnatum*.
Cromwell, R. O. (paper): Fusarium-Blight, or Wilt Disease, of the Soybean..... 421–440
Cronartium—
ribicola—
internal telia..... 330–331
parasite of *Ribes roezli*..... 330
spp., discovery of internal telia produced by..... 329–332
Crowngall—
causal organism..... 165–186
mechanism of tumor growth in..... 165–186
Cucumber. See *Cucumis sativus*.
Cucumis sativus, host plant of *Choanephora cucurbitarum*..... 320
Cucurbita—
pepo, host plant of *Choanephora cucurbitarum*..... 320
spp.—
appearance..... 321
causal organism..... 320
economic importance..... 320
Curly-leaf. Syn. Little-leaf.
Cutworm, parasite of..... 189–194

Diagnosis of Tuberculosis by Complement Fixation, with Special Reference to Bovine Tuberculosis (paper)..... 1–20
Dilatometer—
description..... 197–200
measurement of soil moisture by..... 195–217
principle..... 197
value..... 215

Diphtheria serum. See Serum, diphtheria. Page
Discovery of Internal Telia Produced by a Species of Cronartium (paper)..... 329–332
Dissemination of the Angular Leafspot of Cotton (paper)..... 457–475

Effect of Fertilizers on the Composition and Quality of Oranges (paper)..... 127–138
Effect of Irrigation Water and Manure on the Nitrates and Total Soluble Salts of the Soil (paper)..... 333–359
Eichhorn, A., and Blumberg, A. (paper): Diagnosis of Tuberculosis by Complement Fixation, with Special Reference to Bovine Tuberculosis..... 1–20
Eichhorn, A., Berg, W. N., and Kelser, R. A. (paper): Immunity Studies on Anthrax Serum..... 37–56
Enzym, plant, influence on fermentation of corn silage..... 361–380
Errata..... IV
Euglobulin—
analysis..... 451–454
transformation of pseudoglobulin into... 449–456
Euxoa tessellata, occurrence of *Sorosporella uvella* in..... 190
Ewe, milk. See Milk, ewes'.
Ewes' Milk: Its Fat Content and Relation to the Growth of Lambs (paper)..... 29–36

Fagus sp., host plant of *Phytophthora fagi*... 265
Faulwetter, R. C. (paper): Dissemination of the Angular Leafspot of Cotton..... 457–473
Fertilizer—
effect on little-leaf of vine..... 395–397
effect on quality of *Citrus aurantium*.... 127–138
effect on composition of *Citrus aurantium* 127–138
Fruit trees—
arsenical injury to..... 283–318
control of arsenical injury to..... 295–296
effect of season on arsenical injury to..... 306
systemic poisoning by arsenic..... 313–314
Fry, W. H., Hazen, W., and Shorey, E. C. (paper): Calcium Compounds in Soils..... 57–77
Fungicides, effect on little-leaf of vine..... 394–395
Fusarium—
conglutinans, characters..... 431
discolor, characters..... 430–431
lycopersici, characters..... 430–431
niveum, characters..... 430–431
orthoceras, characters..... 431
radicicola, temperature relations..... 139–164
oxysporum, characters..... 430–431
redolens, characters..... 431
sp. on soybean—
characters..... 430–431
morphological comparison with *Fusarium tracheiphilum*..... 431–433
spp., effect of culture media on..... 428–429
tracheiphilum, characters..... 430–431
vasinfectum, characters..... 430–431
Fusarium-blight of *Soja max*—
history..... 422–424
manner of infection..... 425–427
occurrence..... 422–424
symptoms..... 424–425
Fusarium-Blight, or Wilt Disease, of the Soybean (paper)..... 421–440

Globulin— Page
chemical analyses..... 45–50
inoculation of animals..... 41–45
preparation..... 40–41
Glomerella cingulata, temperature relations. 139–164
Glycine soja. Syn. *Soja max*.
Gossypium—
herbaceum, host plant of *Choanephora cucurbitarum*..... 320
spp., infection of seed..... 462–464
Grape. See *Vitis* spp.
Gypsum, effect on potassium in orthoclase-bearing soils..... 21–28

Harris, F. S., and Butt, N. I. (paper): Effect of Irrigation Water and Manure on the Nitrates and Total Soluble Salts of the Soil..... 333–359
Hazen, W., Shorey, E. C., and Fry, W. H. (paper): Calcium Compounds in Soils.... 57–77
Helicosporium nymphaearum—
casual organism of leafspot-rot of pond lilies..... 219–232
cultural characters..... 225–226
description..... 229–230
germination of sclerotia..... 227
growth..... 227, 228
host-parasite relation..... 228–229
inoculation..... 221–225
isolation..... 220–221
parasite of—
Nelumbium speciosum..... 225
Nymphaea spp..... 225
taxonomy..... 229, 230
temperature relations..... 228
viability..... 227
Hibiscus—
coccineus, host plant of *Choanephora cucurbitarum*..... 320, 326
esculentus, host plant of *Choanephora cucurbitarum*..... 320, 326
syriacus, host plant of *Choanephora cucurbitarum*..... 320, 326
Hog goober. See *Worandzia subterranea*.
Hog serum. See Serum, hog.
Hyperimmunization, change in serum proteins during..... 50–52

Immunity Studies on Anthrax Serum (paper) 37–56
Insect, dissemination of *Bacterium malvacearum* by..... 464–466
Insecticides, effect on little-leaf of vine..... 394–395
Iron arsenate, injury to fruit trees..... 300, 302
Irrigation, effect on nitrates and soluble salts of soil..... 333–359

Kelser, R. A., Eichhorn, A., and Berg, W. N. (paper): Immunity Studies on Anthrax Serum..... 37–56

Lamb, A. R. (paper): The Relative Influence of Microorganisms and Plant Enzyms on the Fermentation of Corn Silage..... 361–380
Lamb, growth—
relation of fat content of ewes' milk to..... 29–36
relation of milk yield to..... 33–35
Lead arsenate, injury to fruit trees. 299, 303, 308–309
Leafspot-rot of *Nymphaea* spp., control.... 230–231
Leafspot-Rot of Pond Lilies Caused by Helicosporium nymphaearum (paper)..... 219–232

Lily, pond. See *Nymphaea*. Page
Lime—
effect on arsenicals 303
effect on potassium in orthoclase-bearing soils 21–28
Little-leaf—
character of soil in relation to 383–388
comparison with other vine diseases 394
distribution 381–383
effect of—
fertilization 395–397
fungicides 394–395
insecticides 394–395
etiology 392–393
characters 381–382
infectiousness 392
loss by 381
occurrence on *Vitis* spp 381–398
symptoms on *Vitis* spp 389–394
variations in attacks 388–389
variations in susceptibility 393
Little-Leaf of the Vine (paper) 381–398

McClintock, J. A. (paper): Peanut Wilt Caused by Sclerotium Rolfsii 441–448
McCray, A. H. (paper): Spore-Forming Bacteria of the Apiary 399–420
Malus sylvestris—
arsenical injury 287–317
temperature relations of apple-rot fungi on 138–163
Man, treatment of anthrax in 52–53
Manure effect on nitrates and soluble salts of soil 333–359
Measurement of the Inactive, or Unfree, Moisture in the Soil by Means of the Dilatometer Method (paper) 195–217
Mechanism of Tumor Growth in Crowngall (paper) 165–186
Microorganism, influence on fermentation of corn silage 361–380
Milk, ewe's—
fat content 29–33
relation to growth of lambs 32–35
Moisture, soil. See Soil moisture.
Morris, H. E., and Swingle, D. B. (paper): Arsenical Injury through the Bark of Fruit Trees 283–318
Morse, W. J. (paper): Studies upon the Blackleg Disease of the Potato, with Special Reference to the Relationship of the Casual Organisms 79–126
Mucor stolonifer, temperature relations 152

Nelumbium speciosum, host plant of *Helicosporium nymphaearum* 225
Neofabraea malicorticis, temperature relations. 139–164
Nitrogen—
amino, change in fermenting corn silage 368–370, 372, 375
ammonia, change in fermenting corn silage. 369–370, 372
effect on composition of *Citrus aurantium*. 132–134
Nymphaea spp.—
host plants of *Helicosporium nymphaearum* 225
leafspot-rot 219–232

Okra. See *Hibiscus esculentus*. Page
Orange. See *Citrus aurantium*.
Orthoclase—
effect of calcium hydrate on potassium in 24
effect of calcium sulphate on potassium in. 24, 27
soils bearing, availability of potassium in 21–28

Paint, protection of fruit-tree wounds by 295–296
Panax quinquefolia, host plant of *Phytophthora cactorum* 259
Peanut. See *Arachis hypogaea*.
Peanut Wilt Caused by Sclerotium Rolfsii (paper) 441–448
Pear. See *Pyrus* spp.
Pegmatite—
effect of calcium hydrate on potassium in 22–23
effect of calcium sulphate on potassium in 22–23
Penicillium expansum, temperature relations. 139–164
Pestalozzia funerea, temperature relations 139–164
Phyllocactus sp., host plant of *Phytophthora cactorum* 259
Phytophthora—
economic importance of genus 234–235
identification of species of 270–271
studies of the genus 233–276
arecae—
growth 238
morphology of the mycelium 241–242
cactorum—
growth 238
morphology of mycelium 241–242
parasite of *Phyllocactus* sp 259
erythroseptica, morphology of mycelium 241–242
faberi—
growth 238
morphology of mycelium 241–242
fagi—
growth 238
morphology of mycelium 241–242
parasite of *Fagus* sp 265
infestans—
association with blackleg of *Solanum tuberosum* 89
growth 238
morphology of—
mycelium 241–242
sexual bodies 263–265
jatrophae—
growth 238
morphology of mycelium 241–242
nicotianae—
growth 238
morphology of mycelium 241–242
parasitica—
growth 238
morphology of mycelium 241–242
phaseoli—
growth 238
morphology of mycelium 241–242
spp.—
biometrical constants 265–270
morphology of—
chlamydospores 255–258
conidia 243–255
conidiophores 242–243
mycelium 241–242
sexual bodies 258–263
spore forms produced by 239–241

Phytophthora—Continued. Page
syringae—
growth ... 238
morphology of mycelium ... 241-242
Plum. See *Prunus* spp.
Pond lily. See *Nymphaea* spp.
Potash. See Potassium.
Potassium—
arsenate, injury to fruit trees ... 298-299
arsenite, injury to fruit trees ... 298-299
availability in orthoclase-bearing soils ... 21-28
effect on *Citrus aurantium* ... 131-132, 134
in orthoclase—
effect of calcium hydrate on ... 24
effect of calcium sulphate on ... 24, 27
in pegmatite—
effect of calcium sulphate on ... 22-23
effect of calcium hydrate on ... 22-23
in soil—
effect of calcium hydrate on ... 25
effect of calcium sulphate on ... 25-27
in wheat, effect of calcium sulphate on ... 27
Potato. See *Solanum tuberosum*.
Protein, estimation of ... 451-455
Prunus spp., susceptibility to arsenical injury ... 304-305
Pseudoglobulin—
separation from immune serum ... 38-41
transformation into euglobulin ... 449-456
Pyrus sp., susceptibility to arsenical injury. 304-305
Rand, F. V. (paper): Leafspot-Rot of Pond Lilies Caused by Helicosporium nymphaearum ... 219-232
Relative Influence of Microorganisms and Plant Enzyms on Fermentation of Corn Silage, The (paper) ... 361-380
Ribes roezli, host plant of *Cronartium ribicola*. 330
Ricinus communis, tumor formation in ... 177-181
Ritzman, E. G. (paper): Ewes' Milk: Its Fat Content and Relation to the Growth of Lambs ... 29-36
Rosenbaum, J. (paper): Studies of the Genus Phytophthora ... 233-276

Scarlet hibiscus. See *Hibiscus coccineus*.
Sclerotinia cinerea, temperature relations ... 139-164
Sclerotium Rolfsii—
causal organism of peanut-wilt ... 441-448
effect on *Trifolium incarnatum* ... 447
seed dissemination ... 444-445
Scorzonera hispanica, occurrence of crowngall. 181
Season, relation to arsenical injury of fruit trees ... 306
Seed, dissemination of wilt of *Arachis hypogaea* by ... 444-445
Serum—
anthrax—
effect of heating on pseudoglobulin ... 499-456
immunity studies ... 37-56
standardization, by complement fixation. 53-55
bovine, tests ... 10-14
degree of fixation with ... 11-14
diphtheria, effect of heating on pseudoglobulin ... 449-456
hog, tests ... 16-17
human, tests ... 17
immune, separation of pseudoglobulin from ... 38-41
preparation of globulin from ... 40-41

Serum—Continued. Page
preparations, chemical analyses of ... 45-50
proteins, changes during hyperimmunization ... 50-52
tetanus, effect of heating on pseudoglobulin ... 449-456
Shorey, E. C., Fry, W. H., and Hazen, W. (paper): Calcium Compounds in Soils ... 57-77
Silage—
analysis of ... 365-366
development of—
acidity ... 368-374, 376-377
alcohol ... 368-373, 376-377
effect of antiseptics on fermentation ... 365
fermentation—
chemical change in ... 366-376
rise of temperature in ... 375-376
formation of carbon dioxid in ... 373-374
influence of microorganisms on fermentation ... 361-380
influence of plant enzyms on fermentation ... 361-380
Smith, E. F. (paper): Mechanism of Tumor Growth in Crowngall ... 165-186
Sodium—
arsenate, injury to fruit trees ... 298-299
arsenite, injury to fruit trees ... 298-299, 313
Soil—
analysis, for calcium ... 67-69
calcium compounds in ... 57-77
character in relation to little-leaf ... 383-388
classification according to color ... 59-65
effect of calcium hydrate on potassium in ... 25
effect of calcium sulphate on potassium in ... 25, 27
effect of irrigation water and manure—
on nitrates of ... 333-359
on soluble salts of ... 333-359
moisture, measurement by dilatometer ... 195-217
orthoclase-bearing, availability of potash ... 21-28
petrographic analysis ... 65-66
Soja—
hispida. Syn. *Soja max*.
max—
characters of *Fusarium* spp. on ... 430-431
economic importance of ... 421-422
growth in inoculated soil ... 433-437
Fusarium-blight of—
history ... 422-424
manner of infection ... 425-427
occurrence ... 422-424
symptoms ... 424-425
Solanum tuberosum—
blackleg of—
association with *Phytophthora infestans* ... 89
studies of ... 79-126
sources of infection with blackleg ... 90-91
Sorosporella uvella—
description ... 191-193
occurrence—
in *Euxoa tessellata* ... 190
in cutworms in America ... 189-194
Sorosporella uvella and Its Occurrence on Cutworms in America (paper) ... 189-194
Soybean. See *Soja max*.
Speare, A. T. (paper): Sorosporella uvella and Its Occurrence on Cutworms in America ... 189-194

Page

Species, new 230
Sphaeropsis malorum, temperature relations 139–164
Spore-Forming Bacteria of the Apiary (paper) 399–420
Squash Disease Caused by Choanephora cucurbitarum, A (paper) 319–328
Studies of the Genus Phytophthora (paper) 233–276
Studies upon the Blackleg Disease of the Potato, with Special Reference to the Relationship of the Causal Organisms (paper) 79–126
Sugar, disappearance in fermentation of corn silage 367–372
Swingle, D. B., and Morris, H. E. (paper): Arsenical Injury through the Bark of Fruit Trees 283–318

Temperature Relations of Apple-Rot Fungi (paper) 139–164
Tetanus serum. See Serum, tetanus.
Tetrastichus bruchophagi—
appearance 279–280
classification 278
description of—
adult 282
larva 280–281
pupa 281–282
discovery 277–278
distribution 277–278
hibernation 279
oviposition 280
seasonal history 282
stages of host showing parasitism 279
parasite of—
importance 282
Bruchophagus funebris 277–282
Tetrastichus bruchophagi, a Recently Described Parasite of Bruchophagus funebris (paper) 277–282
Tragopogon pratensis, occurrence of crowngall on 181
Transformation of Pseudoglobulin into Euglobulin (paper) 499–456
Trees, fruit. See fruit trees.
Trichoderma sp., temperature relations 139–164

Page

Trifolium incarnatum, effect of *Sclerotium Rolfsii* on 447
Tuberculosis—
antigens, preparation 5–10
diagnosis by complement fixation 1–20
Tumor—
formation in *Nicotiana tabacum* 177
growth in crowngall 165–186
Urbahns, T. D. (paper): Tetrastichus bruchophagi, a Recently Described Parasite of Bruchophagus funebris 277–282

Vitis spp., little-leaf of 381–398
Volutella fructi, temperature relations 139–164

Water, irrigation, effect on soluble salts of soil 333–359
Washing, effect of, on arsenical injury 302–303
Wheat, effect of calcium sulphate on potassium in 27
Wilt, soybean. See Fusarium-blight of *Soja max*.
Wilting coefficient, temperature relation to soil moisture 203
Wind, dissemination of *Bacterium malvacearum* by 46–469
Wolf, F. A. (paper): A Squash Disease Caused by Choanephora cucurbitarum 319–332
Worandzia subterranea, resistance to *Sclerotium Rolfsii* 445–447

Yellow-leaf. Syn. Little-leaf.
Young, H. D. (paper): Effect of Fertilizers on the Composition and Quality of Oranges. 127–138

Zea mays, silage of—
change in quantity of amino nitrogen in fermentation 368–369, 372, 375
development of acidity in fermenting 368–374, 376–377
disappearance of sugars in fermentation of 367–372, 376
formation of alcohol in fermenting 368–374, 376–377
relative influence of plant enzyms on the fermentation of 361–380
Zinc arsenite, injury to fruit trees 299–303